

UNITED STATES DISTRICT COURT
MIDDLE DISTRICT OF FLORIDA
JACKSONVILLE DIVISION

UNITED STATES OF AMERICA)
)
 v.) CASE NO. 3:19-cr-00001-TJC-PDB
)
JOHN R. NETTLETON,)
)
Defendant.)

GOVERNMENT’S OMNIBUS RESPONSE
TO DEFENDANT’S PRETRIAL MOTIONS

I. INTRODUCTION

The United States of America, by and through the Department of Justice, Public Integrity Section Acting Chief AnnaLou Tirol, Deputy Chief Todd Gee, and Trial Attorney Peter M. Nothstein, hereby submits its Consolidated Response to Defendant’s Pretrial Motions. Dkts. 31-36.

II. PROCEDURAL BACKGROUND

On January 8, 2019, the Defendant, John R. Nettleton (“Defendant”), then a Captain in the U.S. Navy, was charged by a grand jury sitting in and for the Middle District of Florida in a ten-count Indictment (Dkt. 1) with one count of Obstruction of Justice, in violation of 18 U.S.C. § 1512(b)(3) (Count One); one count of Obstruction of Justice, in violation of 18 U.S.C. § 1512(c)(2) (Count Two); one count of Concealment of Material Facts, in violation of 18 U.S.C. § 1001(a)(1) (Count Three); two counts of Falsification of Records, in

violation of 18 U.S.C. § 1519 (Counts Four and Five); and four counts of False Statement, in violation of 18 U.S.C. § 1001(a)(2) (Counts Six through Ten).

This Court initially set a trial date of May 6, 2019 (Dkt. 14), but at the request of the parties, on March 15, 2019 scheduled the jury trial in this matter to commence on January 6, 2020. Dkt. 27.

On July 22, 2019, the Defendant filed six pretrial motions: (1) Motion for Leave to File a Jury Questionnaire and for Individual Voir Dire (Dkt. 31); (2) Motion to Exclude the Indictment from Being Given to Jurors (Dkt. 31); (3) Motion to Strike (Dkt. 33); (4) Motion to Dismiss Count One or Two (Dkt. 34); (5) Motion to Suppress Statements (Dt. 35); and (6) Motion in Limine (Dkt. 36).

The Government agrees to strike the portion of the Indictment which is the subject of the Motion to Strike (Dkt. 33), but opposes the rest of the Defendant's motions.

III. FACTS

The charges in this case stem from the death and investigation into the death of Christopher M. Tur ("Tur"), a civilian, at the Naval Air Station, Guantanamo ("GTMO") in Guantanamo Bay, Cuba on or about January 9, 2015. Tur died after an altercation with the Defendant at the Defendant's home regarding Tur's accusation that the Defendant was engaged in an

extramarital affair with Tur's wife ("Tur's Spouse").

The Defendant served as the Commanding Officer ("Commander") of GTMO from June 2012 until he was relieved of command on January 21, 2015. As the Commander of GTMO, Nettleton had responsibility to ensure the safety and well-being of persons under his command at GTMO. Dkt. 1 at ¶ 5. GTMO is part of the Navy Region Southeast ("NRSE"), which is based at Naval Air Station Jacksonville, located in Jacksonville, Florida. *Id.* at ¶ 3. As the Commander of GTMO, the Defendant reported to the Commander of the Navy Region Southeast ("CNRSE") and the members of the command staff. *Id.* In January 2015, the CNRSE was Rear Admiral M.J. and her Chief of Staff was Captain C.G. *Id.*

Tur, Tur's Spouse, and their children moved to GTMO in May 2011. *Id.* at ¶ 4. Tur was employed as the Loss Prevention Safety Manager at the Navy Exchange, a general store serving the GTMO community. *Id.*

In the early evening of January 9, 2015 there was a "Hail and Farewell" party at the "Hanger Bar" in the basement of the GTMO Officer's Club, which is also known as the "Bayview," to greet the new incoming GTMO Executive Officer ("XO"), and to say goodbye to the outgoing XO. *Id.* at ¶ 16.

According to many accounts, the Defendant, Tur, and Tur's Spouse each consumed a significant amount of alcohol at the Hail and Farewell, and the

Defendant and Tur's Spouse spent time near each other in view of other guests, including Tur. *Id.* at ¶ 17. At approximately 10:00 p.m., outside of the Bayview, Tur yelled at the Defendant and Tur's Spouse, accusing them of having an extramarital affair. *Id.* at ¶ 18. The new XO interceded and convinced the Defendant to go to his house, which was just a short distance away at the end of the peninsula on which the Bayview is located, known as "Deer Point." *Id.*

Shortly after the Defendant returned to his residence, Tur arrived at the Defendant's residence and continued his accusations. The altercation between the Defendant and Tur vacillated between conversation, confrontation and violence. At one point, Tur called a friend and stated that he was "at the Skipper's house" and that he had "just knocked the Skipper out." *Id.* at ¶ 20. The friend was able to hear the Defendant stating words to the effect that Tur had "just knocked him out." *Id.* Later, the Defendant's teenage daughter—the only other person in the Defendant's home that night—heard from her upstairs bedroom shouting and sounds of a physical altercation coming from the floor below. *Id.* at ¶¶ 21-27. At one point, she went to the ground floor and saw Tur standing over her father, who was lying on the kitchen floor. *Id.* at ¶ 22. Later that evening, after the sounds of fighting had stopped, the Defendant came into his daughter's room without a shirt on, and she did not

notice any injuries or blood on him. *Id.* at ¶ 28.

Forensic testing conducted after a search of the Defendant's home between January 20 and 24 by agents of the Navy Criminal Investigative Service ("NCIS"), pursuant to a Command Authorized Search and Seizure, revealed Tur's blood in several rooms of the first floor of the Defendant's house, as well as on the walkway to the Defendant's boat dock. *Id.* at ¶¶ 48, 58. The entrance to the walkway is located a short distance from the side door of the Defendant's home where Tur's blood was found. NCIS agents also recovered a paper towel with Tur's blood on it in the bushes next to the walkway to the dock. *Id.* at ¶ 48.

The next morning, January 10, Tur's Spouse could not find Tur, so she and the friend who Tur had called to say he had "knocked out" the Defendant contacted the Defendant to see if knew where Tur had gone after the Defendant's house. *Id.* at ¶ 29. The Defendant said that he did not know, but did not tell Tur's Spouse or the friend that Tur had come inside and that there had been a fight that left Tur injured and bloody. *Id.* Over the course of January 10 and into January 11, the GTMO Security Department conducted a search for Tur. *Id.* at ¶¶ 30-46. NCIS also began a law enforcement investigation into Tur's disappearance. At approximately 11:00 a.m. on January 11, Tur's body was found floating in the waters just off the coast of

GTMO, nearly in Cuban water. *Id.* at ¶ 47. At that point, NCIS's investigation became a death investigation.

An autopsy of Tur's body conducted on January 13 concluded that the cause of Tur's death was drowning, but that before he died, he suffered broken ribs and soft tissue damage. *Id.* at ¶ 55. Tur also had a laceration on his head. *Id.*

The charges against the Defendant stem from his concealment of material facts and affirmative statements he made to other Navy officers and personnel from approximately the morning of January 10 through January 15, which are detailed in the Indictment (*id.* at ¶¶ 30-56) as well as in Section VIII.A below, insofar as they are relevant to the Motion to Suppress Statements.

On January 21, the Defendant was removed from command by Rear Admiral M.J.

IV. MOTION FOR JURY QUESTIONNAIRE [DKT. 31]

The Defendant has asked this court for the “opportunity to 1) submit a proposed juror questionnaire to be provided to all potential jurors, and 2) participate in individual *voir dire* for purposes of questioning potential jurors about issues related to pre-trial publicity and the potential jurors' views on military service.” Dkt. 31 at 2.

It is the Government's understanding that this Court's regular practice

with regard to jury selection is (1) to allow both parties to propose *voir dire* questions to be asked of the venire by the Court; (2) for the Court to ask the *voir dire* questions of the potential jurors; and (3) for the Court to ask—or allow counsel to suggest—follow up questions in the Court’s discretion that may be asked at the bench where they concern sensitive topics. It is further the Government’s understanding that this practice is followed even in cases that have garnered media attention. *See, e.g.* Order Scheduling Trial (Dkt. 103), Ex. A, *United States v. Corrine Brown*, 3:16-cr-00093-TJC-JRK (Apr. 13, 2017). To the extent the Defendant is requesting that this Court deviate from its regular practice, the Government opposes such deviation.

This cases has garnered some media coverage, but not, as the Defendant argues, a “high level” of media coverage that will result in a “very high likelihood that a substantial number of persons brought in as potential jurors will have some knowledge about this case.” Dkt. 31 at 2. Further, it may be true that members of the venire have “preconceived ideas about military service and the obligations of servicemembers,” but this Court’s usual *voir dire* practices can effectively detect any such preconceived ideas or whether any juror has prejudged the case based on media reports. *Id.* Moreover, the Defendant will be free to suggest *voir dire* questions designed to expose any prejudices or biases of the members of the venire.

For these reasons, the Defendant's Motion For Jury Questionnaire (Dkt. 31) should be denied.

V. MOTION TO PREVENT INDICTMENT FROM GOING TO JURY [DKT. 32]

The Defendant argues in his motion that a copy of the indictment should not be provided to the jury during their deliberations because it is a “‘speaking indictment’ that goes beyond merely reciting the bare-bones facts necessary to meet the elements of the charged offense, but instead gives substantial play-by-play details of the Government’s allegations,” and therefore “allowing the jurors to receive a copy of the indictment provides the Government an unfair advantage over Defendant in that the jurors are presented with a one-sided recitation of the prosecution’s case which they can review and refer back to when deliberating.” Dkt. 32 at 1.

It is established precedent in the Eleventh Circuit and throughout the nation that it is within a trial court’s discretion to provide a copy of the indictment to the jury where the court determines that the indictment would help to organize deliberations and the court instructs the jury that the indictment is not evidence. Given the complicated and fact-intensive nature of the charges against the Defendant, the number of charges, and the expected length of the trial, the Government submits that the Court can and should send a copy of the indictment to the jury during deliberations.

A. Legal Standard

As a “general rule,” a “trial court may, in the exercise of discretion, allow the indictment to be taken into the jury room.” *Bruce v. United States*, 351 F.2d 318, 320 (5th Cir. 1965). When providing the jury with a copy of the indictment, the court should also instruct the jury that the indictment is not evidence. *See United States v. Haynes*, 573 F.2d 236, 242 (5th Cir. 1978) (“The jury was properly instructed that the indictment itself did not constitute evidence, and the indictment contains no inflammatory or pejorative language that would create any prejudice against the accused”). Circuit precedent has even upheld providing a copy of the indictment to the jury *before* trial where a proper cautionary instruction was given. *United States v. Tucker*, 526 F.2d 279 (5th Cir. 1976). Other circuits are in agreement that an indictment can be provided to a jury, along with a cautionary instruction that the indictment is not evidence. *See, e.g., United States v. Press*, 336 F.2d 1003, 1016 (2d Cir. 1964), cert. denied, 379 U.S. 965 (1965) (“Indeed in protracted cases involving numerous counts such as this one, reference to the indictment often serves as a helpful guide in delineating the issues the jury may be called on to decide.”); *United States v. Todaro*, 448 F.2d 64, 66 (3d Cir. 1971).

B. Providing a Copy of the Indictment to the Jury Would Aid in Its Deliberations.

As detailed in the Indictment, the Defendant is charged with ten counts of obstruction of justice, concealment, falsification of records, and false statements that relate to a series of specific actions he took, mostly, over a compressed period of time from January 9, 2015 to January 21, 2015, to conceal his knowledge of the circumstances surrounding Tur’s disappearance and death. The indictment will provide the jury with this chronological series of events—all of which the Government expects will be proven at trial—in a concise, organized format that will help it to organize deliberations. *See* Dkt. 1 at ¶¶ 16-59. Additionally, the indictment will provide the jury with a clear description of the charged obstruction, concealment, and false statements, as well as the actions the Defendant undertook and how those actions fit into that lengthy chronological narrative. Compare *id.* ¶¶ 61, 63, 65, 67, 69, 71, 73, 75, 77, 79 with ¶¶ 16-59. In a trial that will involve numerous witnesses and likely last for several weeks, an outline of the charges will be of great assistance to the jury in its deliberations.

The Defendant argues that providing the indictment to the jury would provide them with only “one party’s – the Government’s – account of the disputed events,” which would be a “significant procedural advantage available only to the Government.” Dkt. 32 at 2. This argument has been considered

by other courts and rejected. *See, e.g., Todaro*, 448 F.2d at 66 (these complaints are answered by “a limiting instruction that the indictment does not constitute evidence, but is an accusation only”). Indeed, concerns about prejudice have so little merit in deciding whether to provide a jury with the indictment that one court of appeals concluded that the district court did not commit plain error when it provided the jury with an unredacted copy of an indictment that contained inadmissible and prejudicial information about the defendant. *United States v. Roy*, 473 F.3d 1232 (D.C. Cir. 2007).

For these reasons, this Court should deny the Defendant’s Motion to Prevent Indictment from Going to the Jury. Dkt. 32.

VI. OPPOSITION TO MOTION TO STRIKE [DKT. 33]

In light of recent court decisions not binding on this court, the government agrees to strike the language “the Department of the Navy investigation into the circumstances surrounding the disappearance, injury, and death of Tur” in Count Two of the Indictment. Dkt. 1 at ¶ 63; *see United States v. Young*, 916 F.3d 368 (4th Cir. 2019); *United States v. Sutherland*, 921 F.3d 421 (4th Cir. 2019).

VII. OPPOSITION TO MOTION TO DISMISS [DKT. 34]

The Defendant argues in his Motion to Dismiss (Dkt. 34) that (1) Counts One and Two, which charge violations of 18 U.S.C. §§ 1512(b)(3) and

1512(c)(2), respectively, are multiplicitous, (2) that the Court should therefore dismiss one of these two counts or order the Government to choose between them, and (3) that the Court should also dismiss Counts Three through Ten, which charge violations of § 1001(a)(1) (Count Three), § 1519 (Counts Four and Five), and § 1001(a)(2) (Counts Six through Ten). Dkt. 34 at 4-5. Other than discussing the definition of multiplicity and the potential remedies when it is found, the Defendant provides no explanation as to why Counts One and Two are multiplicitous, or why Counts Three through Ten should be dismissed, beyond the following:

Here, the Government has alleged that Defendant committed the exact same acts in Counts One and Two, and portions of that same conduct in Counts Three through Ten. There are no acts alleged in Counts Three through Ten that are not already encompassed by Counts One and Two. The Government shouldn't be able to argue to the jury that Defendant committed ten separate crimes based on the exact same course of conduct which the Government could charge in one single count. The manner in which the Government has charged Defendant raises the risk of jury confusion and a compromised verdict.

Id.

The Defendant's arguments are without merit and should be rejected. Counts One and Two are not multiplicitous; they charge different offenses with different elements, thereby satisfying the standard for avoiding multiplicity set

forth in *Blockburger v. United States*, 284 U.S. 299, 304 (1932). Additionally, although some of the conduct charged in Counts Three through Ten overlaps with conduct charged in Counts One and Two, there is no legal prohibition on the Government's or grand jury's power to charge the same conduct in multiple ways in the same indictment provided that the offenses have different elements.

The Defendant further argues that Counts Six through Nine, which charge false statements made in violation of 18 U.S.C. § 1001(a)(2), should be dismissed because they are lesser-included offenses of Count Three, which charges a scheme to falsify and conceal material facts in violation of 18 U.S.C. § 1001(a)(1). Dkt. 34 at 5-7. This argument should also be rejected, as the Eleventh Circuit and other courts have held that Sections 1001(a)(1) and (a)(2) are two distinct offenses that proscribe different conduct.

A. Legal Standard

1. Multiplicity

“An indictment is multiplicitous if it charges a single offense in more than one count.” *United States v. Williams*, 527 F.3d 1235, 1241 (11th Cir. 2008) (citing *Ward v. United States*, 694 F.2d 654, 660–61 (11th Cir. 1983)). “A multiplicitous indictment not only subjects the Defendant to numerous sentences for one offense, but also ‘prejudice[s] the Defendant and confuse[s] the jury by suggesting that not one but several crimes have been committed.’”

Williams, 527 F.3d at 1241 (quoting *United States v. Hearod*, 499 F.2d 1003, 1005 (5th Cir.1974) (*per curiam*)) (footnote omitted). “A multiplicitous indictment therefore violates the principles of double jeopardy because it gives the jury numerous opportunities to convict the Defendant for the same offense. We use the *Blockburger* test to determine whether an indictment is multiplicitous, verifying that each count requires an element of proof that the other counts do not require.” *Williams*, 527 F.3d at 1241 (citing *Ward*, 694 F.2d at 661).

2. Lesser-Included Offenses

In *Schmuck v. United States*, the Supreme Court outlined the “elements test” for defining a lesser-included offense. 489 U.S. 705, 720–21 (1989). One offense is only included within another if all of the elements of the lesser offense comprise a subset of those required in the greater offense. *Id.* The “elements test” looks only at the legal elements; the factual allegations in a case play no role in this analysis. *See id.* at 720–21. “[T]he elements approach involves a textual comparison of criminal statutes,” and “promotes judicial economy by providing a clearer rule of decision and by permitting appellate courts to decide whether jury instructions were wrongly refused without reviewing the entire evidentiary record for nuances of inference.” *Id.*

B. Counts One and Two Are Not Multiplicitous and the Government Does Not Need to Choose Between Those Counts, Nor Should the Court Dismiss Counts Three Through Ten Based on the Overlap of Charged Conduct with Counts One and Two.

A simple review of the elements of Sections 1512(b)(3) and 1512(c)(2) demonstrates that an indictment charging both offenses, like the one in this case, is not multiplicitous.

To violate Section 1512(b)(3), “a defendant must knowingly and willfully (1) engage in misleading conduct toward another person, (2) with the intent to hinder, delay or prevent the communication of information to a federal official, (3) about the commission or the possible commission of a federal crime.” *United States v. Ronda*, 455 F.3d 1273, 1284 (11th Cir. 2006) (citing *United States v. Veal*, 153 F.3d 1233, 1253 (11th Cir. 1998), cert. denied, 526 U.S. 1147 (1999)).

To prove a violation of Section 1512(c)(2), the Government must prove that: (1) there was an official proceeding taking place; (2) the Defendant engaged in conduct which constituted a substantial step toward the commission of the crime of obstruction of an official proceeding; (3) the Defendant acted corruptly, *i.e.*, with an improper purpose and to engage in conduct knowingly and dishonestly with the specific intent to subvert, impede or obstruct the proceeding; and (4) the natural and probable effect of the Defendant’s conduct would be the interference with the due administration of justice. *United States*

v. Mintmire, 507 F.3d 1273, 1289 (11th Cir. 2007); *see also United States v. Friske*, 640 F.3d 1288, 1291 (11th Cir. 2011).

Plainly, there are multiple elements of both offenses that are not included in the elements of the other. For example, a violation of Section 1512(c)(2) requires an official proceeding be foreseeable, which is not required by Section 1512(b)(3). The two offenses clearly satisfy the *Blockburger* test to avoid being multiplicitous or violating the Double Jeopardy Clause when charged in the same indictment. *See Williams*, 527 F.3d at 1241.

For the same reasons, this Court should reject the Defendant's more general argument that the Court should dismiss Counts Three through Ten—for which he provides no citations to authority—because “[t]he Government shouldn’t be able to argue to the jury that Defendant committed ten separate crimes based on the exact same course of conduct which the Government could charge in one single count.” Dkt. 34 at 5. Indeed, if the Government proves the allegations in the Indictment to the jury beyond a reasonable doubt, the Defendant *did* commit ten separate crimes. There is no general prohibition created by the Double Jeopardy Clause or some other constitutional principle on charging the same or similar conduct in multiple counts that have different elements, as evidenced by the existence of the *Blockburger* test itself.

Moreover, to the extent that the Defendant's argument may be that

Counts Three through Ten are multiplicitous of either Counts One or Two, that argument would also have no merit. Subsection (a)(1) of 18 U.S.C. § 1001, which is charged in Count Three, has elements different from subsection (a)(2), which is charged in Counts Six through Ten. Both subsections contain elements different from Sections 1512(b)(3) and (c)(2). Additionally, falsification of records, in violation of 18 U.S.C. § 1519, which is charged in Counts Four and Five, has different elements than all of the other offenses.

C. The False Statement Charges in Counts Six to Nine Are Not Lesser-Included Offenses of the Concealment in Count Three Because Each Offense Involves Separate Elements.

The Defendant's argument that false statements made in violation of 18 U.S.C. § 1001(a)(2) are lesser-included offenses of violations of 18 U.S.C. § 1001(a)(1) has been rejected by the Eleventh Circuit, which held that Section 1001 "encompasses two distinct offenses, false representation and concealment of a material fact." *United States v. Tobon-Builes*, 706 F.2d 1092, 1096 (11th Cir. 1983). Numerous other circuits have held the same. *See, e.g., United States v. Shaw*, 150 F. App'x 863, 877 (10th Cir. 2005); *United States v. Anzalone*, 766 F.2d 676, 682 (1st Cir. 1985); *United States v. Diogo*, 320 F.2d 898, 902 (2d Cir. 1963); *United States v. Kenny*, 236 F.2d 128 (3d Cir. 1956).

Courts have held as much because Section 1001 "proscribes two different types of conduct: concealment of material facts and false representations."

United States v. Mayberry, 913 F.2d 719, 722 n.7 (9th Cir. 1990). In other words, the two offenses satisfy the *Schmuck* standard because a defendant can perform either action without necessarily performing the other. While the “objective of both offenses may be the same, to create or foster on the part of a Government agency a misapprehension of the true state of affairs. . . . What must be proved to establish each offense . . . differs significantly.” *Diogo*, 320 F.2d at 902. “False representations . . . require proof of actual falsity; concealment requires proof of willful nondisclosure by means of a ‘trick, scheme or device.’” *Id.*; see also *Mayberry*, 913 F.2d at 722 n.7 (identifying the same distinction). Furthermore, a “conviction under § 1001(a)(1), unlike that under § 1001(a)(2), requires proof that the Defendant had a legal duty to disclose the fact concealed.” *United States v. Curran*, 20 F.3d 560, 566 (3d Cir. 1994).

As part of his lesser-included offense argument, the Defendant maintains that each subsection of 18 U.S.C. § 1001 “effectively requires the Government to prove the Defendant willfully and knowingly made false statements,” and he notes that the false statements alleged in Counts Six through Nine are also alleged as actions taken during the falsification and concealment scheme charged in Count Three. Dkt. 34 at 6-7. In so arguing, the Defendant ignores that Count Three alleges that he took other steps to conceal material facts besides making these false statements, such as concealing from Navy personnel

that he and Tur had engaged in a physical altercation at his residence that left Tur injured. *See* Dkt. 1 at ¶ 65.

Most importantly, the Defendant's analysis wholly misses the point that there are elements of Section 1001(a)(2) false statement offenses that are not included in Section 1001(a)(1) concealment offenses, and vice versa. As the Eleventh Circuit stated in *Tobon-Builes*, "[f]alse representations under § 1001 require proof that the Defendant knowingly made a false or fraudulent statement; concealment requires proof of willful nondisclosure by means of a trick, scheme, or device. Generally, concealment violations under § 1001 relate to the nondisclosure of statements required by statute, government regulation or form." 706 F.2d at 1096–97 (internal quotation marks and citations omitted). Accordingly, § 1001(a)(2) offenses cannot be lesser-included offenses of § 1001(a)(1) offenses. *Schmuck*, 489 U.S. at 720–21.

The Defendant's arguments should therefore be rejected and the Defendant's Motion to Dismiss (Dkt. 34) should be denied.

VIII. OPPOSITION TO MOTION TO SUPPRESS STATEMENTS [DKT. 35]

The Defendant's Motion to Suppress Statements seeks to suppress all of the Defendant's statements made to Rear Admiral M.J. and Captain C.G. beginning on January 12, 2015. Dkt. 35 at 3. In the motion, the Defendant acknowledges that Article 31 of the Uniform Code of Military Justice (UCMJ)

does not apply in federal court and that the Defendant was not in “custody” for purposes of *Miranda v. Arizona*, 384 U.S. 436 (1966), but argues that the alleged failure of Rear Admiral M.J. and Captain C.G. to advise the defendant of his Article 31 or *Miranda* rights, coupled with the “subtle pressures” and “subtle coercion” associated with questioning by military superiors, renders the statements involuntary. Dkt. 35 at 7. The defendant’s motion should be rejected because (1) Article 31 does not apply in federal court, but even if it did, Navy personnel did not violate the Defendant’s Article 31 rights; (2) the Defendant’s *Miranda* rights were not violated because he was never in custody or being interrogated; and (3) the Defendant’s statements were all voluntary.

A. Additional Facts Regarding The Defendant’s Statements.

What follows are additional facts from January 2015 relating to the statements that the Defendant seeks to suppress.

1. January 12

On January 12 Rear Admiral M.J. and Captain C.G. “learned from the Navy Inspector General’s Office that an anonymous complaint had been filed, alleging that ‘NETTLETON and Tur’s Spouse had an extramarital affair and were engaging in physical contact at the Bayview during the Hail and Farewell party on or about January 9, 2015.’” Dkt 1 at ¶ 52; Ex. A (excerpts from Captain C.G. grand jury testimony) at 33 (citation to original pagination); Ex.

B (statement of Admiral M.J.) at 2. Captain C.G. testified before the grand jury that when he learned of this anonymous complaint, he spoke with the Inspector General (IG) about initiating an investigation into the complaint to “verify or . . . disprove the veracity of the claims.” Ex. A at 34.

Later that day, Captain C.G. spoke with the Defendant, but did not inform him of the complaint because he “didn’t want to taint the . . . investigation.” Ex. A at 34. Captain C.G. explained that he and Rear Admiral M.J. were superior to the Defendant in the chain of command, “[s]o if there were a disciplinary issue for him, then we would be responsible for administering military justice against Captain Nettleton.” *Id.* at 35. Captain C.G. explained that because of that role in the potential disciplinary process,

I had to be very careful in my discussions with him, because if I were to have begun probing [Nettleton] without reading him those types of rights, then that information—any information that I might have asked him or that he might have revealed, I wouldn’t have been able to use then in a subsequent military . . . disciplinary proceeding. So I had to be very careful.

Id. Captain C.G. said that because of his concerns, he was not “probing [Nettleton] asking him a whole bunch of questions.” *Id.* at 35-36. Admiral M.J. did not speak with the Defendant on January 12. Ex. B at 2.

2. January 13

On Tuesday, January 13, Captain C.G. received what he referred to as a

“strange call” from the Defendant. Ex. A at 36. Captain C.G. said that Nettleton “seemed to be a little bit rattled” and told Captain C.G., “I want to let you know there’s some crazy rumors.” *Id.* The Defendant explained: “I was out and about doing my work in the community and a couple of folks that I know stopped me and said there’s these crazy rumors about me having an affair with the wife of this individual who showed up dead.” *Id.* The Defendant continued, stating “you know, rumors spread quickly here, but, . . . I want you to know there’s absolutely no truth to this rumor. I was not having an affair. None of this is going on.” *Id.*; Dkt. 1 at ¶ 53. The Defendant told Captain C.G. that the rumors that he was having an affair with Tur’s Spouse “unequivocally” were not true. Ex. A at 37.

Captain C.G. perceived that the Defendant “was a little bit rattled” so in his “role as chief of staff and mentor,” he told the Defendant, “JR, you can’t really worry about this stuff. You’ve got a lot to take care of. You’ve got to take care of this family. You’ve got a lot of work to do to organize getting folks there.” *Id.* at 40. Captain C.G. explained that the comment about “getting folks there” was in reference to the additional NCIS agents and Tur family members who needed to be transported to GTMO for the investigation and the funeral. *Id.* at 40.

Captain C.G. continued:

Now there's an investigation going on. You know, you're telling me it's not true. Great. That's all going to come out in the wash in this investigation. I said, you can't really focus on that right now. You need to focus on doing your job as the commanding officer and make sure you're doing everything you can to not only take care of the family, but, you know, you have a wider community now. There's a 5,000 person community there that's very isolated, very insular, and close that you need to insure that the community is taken care of as well.

Id.

Also on January 13, Rear Admiral M.J. directed the Defendant to call her. Ex. B at 3. Rear Admiral M.J., also concerned about questioning the Defendant, did not ask him directly about the incidents of January 9, and instead asked the Defendant only for a status update. During this phone call, the Defendant volunteered to Rear Admiral M.J., for the first time, that Tur had gone missing on or about January 9 after attending a command event earlier in the evening, the Hail and Farewell party at the Bayview, at which the Defendant had been present. Ex. B at 3; Dkt. 1 ¶ 54. Rear Admiral M.J. directed the Defendant to report that information to NCIS. Ex. B at 3. Rear Admiral M.J. did not consider this instruction to be a direct order, but expected that he would have contacted NCIS as she directed. *Id.*

3. January 14

According to Captain C.G., on Wednesday January 14 or Thursday

January 15, the Defendant called him and said “I need to tell you something” and “I wanted to provide some additional information.” Ex. A at 43. The Defendant went on to tell Captain C.G. that the previous Friday, January 9, there had been a going away party at the Officer’s Club attended by several people, including himself, Tur, and Tur’s Spouse. *Id.* The Defendant said that during the event, he had gotten into a verbal altercation with Tur over Tur’s accusations that the Defendant was having an affair with Tur’s Spouse. *Id.* The Defendant also said that Tur later came to the Defendant’s house, where Tur, standing at the front door to the house, repeated the accusations, which the Defendant denied. *Id.* at 44. The Defendant said that Tur had wanted to come into the Defendant’s house, but that the Defendant would not allow him to enter because the Defendant’s daughter was home. *Id.* The Defendant explained that at some point, Tur left the Defendant’s house. *Id.*

Captain C.G. stated that during this conversation, Nettleton was providing him with new facts regarding January 9, which “absolutely shocked” Captain C.G. because the facts were “180 degrees out from what [the Defendant] had been providing.” *Id.* at 45. Captain C.G. said that his “jaw dropped to my belt line” after hearing the new information. *Id.* At no point did the Defendant tell Captain C.G. that Tur came into his home or that there was a physical altercation between the men in the home. *Id.* at 46.

During the conversation, Captain C.G. stated that “as an inquisitive person” he “wanted to get some of the details, but I couldn’t do that because I would have been soliciting information that would then would have been tainted for any type of military justice.” *Id.* at 47. As a result of this concern, he was “very careful” during the conversation not to ask questions of the Defendant. *Id.*

4. January 15-16

Later in the week, either Thursday or Friday, the Defendant made another “strange call” to Captain C.G. *Id.* at 51. The Defendant said to Captain C.G. that “this sheds a bad light on me as the CO. It sheds a bad light on the Navy because of these untrue rumors that are going around. . . . I know how it looks. None of it is true.” *Id.* The Defendant said, “maybe I should resign; maybe you guys should just let me resign as commanding officer and, you know, kind of walk away from this thing and that way it doesn’t shed a bad light on the command and on the Navy. *Id.* at 51-52. Captain C.G. replied that, “it is what it is at this point. There’s an investigation underway. The investigation has to play out now, and so for you to resign really would be moot. We have to just let it play out and see where it goes.” *Id.* at 52.

5. January 20

On Tuesday, January 20, NCIS agents attempted to interview the

Defendant. At the beginning of the interview, an agent presented to him the written advisement of the offenses of which he was suspected, pursuant to the UCMJ. After the agent read the written advisement form's first two charges, which stated that the Defendant was "suspected of adultery and involvement in Chris Tur's death," the agent asked if the Defendant "understands everything on there?" The Defendant responded "I do but, uh," and then the agent said, "This doesn't mean you did anything, it is a formality." The Defendant said, "I know I haven't committed any of this, but I'm going to go ahead and invoke my rights right now if you are going to accuse me of adultery and involvement in the death of Chris Tur. Obviously I'm not going to continue this interview. . . . I'd like to have an attorney present." The interview was then terminated.

Also on January 20, Rear Admiral M.J. made the decision to relieve the Defendant of his command of GTMO. Ex. B at 4. After being relieved of command the next day, January 21, the Defendant had a separate conversation with Captain C.G. in which he asked if it would be possible to resign instead of being relieved, as he had offered to do the week before. Ex. A at 53-54. Captain C.G. replied that it would not be possible. *Id.* at 54.

6. January 21

On January 21, 2015, Captain C.G. arrived at GTMO to assume command of the air station. He had a brief conversation with the Defendant,

during which the “tone of the conversation was . . . keep your head up.” Ex. A at 54. Captain C.G. said to the Defendant, “if what you’re saying is true, none of this is true, then it’s all going to come out and you’ll be exonerated.” During this brief conversation, the Defendant did not provide any additional information relating to his interactions with Tur. *Id.* at 55.

B. Legal Standard

The Defendant’s sole basis to exclude the statements made by the Defendant to Rear Admiral M.J. and Captain C.G. from January 12 and thereafter is that those statements were involuntary “due to the inherent ‘subtle pressures’ and ‘subtle coercion’ of their military relationship.” Dkt. 35 at 7. The Defendant concedes that neither Article 31, because “courts have declined to apply the Article 31 exclusionary rule in federal criminal proceedings,” nor *Miranda*, because the Defendant “was not ‘in custody’ for purposes of *Miranda*,” are grounds to suppress any statements by the Defendant. Dkt. 35 at 4. Nevertheless, the Defendant argues by analogy that the Defendant’s statements were involuntary because neither Article 31 nor *Miranda* were complied with. Dkt. 35 at 10-13.

1. Article 31 is not a Basis to Exclude Statements In Federal Court, and Even if it was, the Defendant’s Article 31 Rights were not Violated.

Under Article 31 of the UCMJ, no person may

request any statement from, an accused or a person suspected of an offense without first informing him of the nature of the accusation and advising him that he does not have to make any statement regarding the offense of which he is accused or suspected and that any statement made by him may be used as evidence against him in a trial by court-martial.

10 U.S.C. § 831(b). Military courts have stated that rights warnings are required if “(1) the person being interrogated is a suspect at the time of the questioning, and (2) the person conducting the questioning is participating in an official law enforcement or disciplinary investigation or inquiry.” *United States v. Swift*, 53 M.J. 439, 446 (C.A.A.F. 2000).

Whether a person is a suspect is an objective question that “is answered by considering all the facts and circumstances at the time of the interview to determine whether the military questioner believed or reasonably should have believed that the servicemember committed an offense.” *Id.* (citing *United States v. Good*, 32 MJ 105, 108 (CMA 1991)). Information must rise to the level of being more than a “hunch” to designate a servicemember as a suspect. *Swift*, 53 M.J. at 447.

Military case law “recognizes the difference between questioning focused on the accomplishment of a military mission, including an administrative function, and questioning to elicit information for use in disciplinary proceedings. *Id.* at 446 (citing *United States v. Brown*, 40 MJ 152, 154 (CMA

1994); *United States v. Shepard*, 38 MJ 408 (CMA 1993)). Whether the questioning was part of a law enforcement or disciplinary investigation “is determined by assessing all the facts and circumstances at the time of the interview to determine whether the military questioner was acting or could reasonably be considered to be acting in an official law-enforcement or disciplinary capacity.” *Swift*, 53 M.J. at 446 (internal citations omitted).

Article 31, by its terms, is “directed solely to the . . . suppression of evidence in court-martial proceedings, and [does] not purport to apply to federal court proceedings. *United States v. Newell*, 578 F.2d 827, 833 (9th Cir. 1978); *United States v. Singleton*, 600 F.2d 553, 555 (5th Cir. 1979) (“Article 31(b) by its terms is limited to evidence used in a trial by court-martial.”); 10 U.S.C. § 831(d) (“No statement obtained from any person in violation of this Article . . . may be received in evidence against him in a trial by court-martial.”); *see also Burns v. Wilson*, 346 U.S. 137, 140 (1953) (“Military law, like state law, is a jurisprudence which exists separate and apart from the law which governs in our federal judicial establishment.”). Unlike *Miranda*, the “primary source of the [Article 31] rights warning requirements in military law is a statutory enactment, not constitutional adjudication.” *Swift*, 53 M.J. at 445. As an example, the protections of Article 31 must be read even when a suspect is not in custody. *United States v. Baird*, 851 F.2d 376, 383 (D.C. Cir. 1988).

The Defendant concedes that Article 31 does not apply in federal court, but argues that one reason the Defendant's statements were involuntary was that the Defendant was improperly not advised of his Article 31 rights. This is not the case; the statements by the Defendant to Rear Admiral M.J. and Captain C.G. from January 12 and thereafter were not obtained in violation of Article 31. Assuming for present purposes that Rear Admiral M.J. and Captain C.G. suspected, or reasonably should have suspected, the Defendant of violations of the UCMJ, the record shows that both Rear Admiral M.J. and Captain C.G. were cognizant of the Defendant's rights and so did not question him regarding his potential exposure.

Most of the Defendant's statements involve information he volunteered during conversations that the Defendant initiated. *See* Ex. A at 36, 43. Any questions asked by Rear Admiral M.J. and Captain C.G. were administrative questions focused on the "accomplishment of a military mission," such as questions intended to obtain status updates, the welfare of the air station, and then later the arrangements for Tur's funeral and his family's travel to GTMO. *See Swift*, 53 M.J. at 446. The Defendant does not allege, and has provided no evidence to suggest, that either Rear Admiral M.J. or Captain C.G. asked the Defendant questions intended "to elicit information for use in disciplinary proceeding." *Id.* Although Article 31 is not a basis to suppress the statements,

to the extent that the Navy's compliance with Article 31 is relevant to the Court's determination of the voluntariness of the Defendant's statements, the record does not establish a violation of Article 31.

2. The Defendant's *Miranda* Rights Were Not Violated.

Likewise, the Defendant argues that the fact that he was not advised of his *Miranda* rights is a factor showing that his statements were involuntary, Dkt. 35 at 10, but the Defendant's *Miranda* rights were not violated. A member of the military subject to custodial interrogation is entitled to be advised of his right against self-incrimination and right to representation by an attorney, just a civilian. *United States v. Rogers*, 659 F.3d 74, 78 (1st Cir. 2011); *see Garcia v. Singletary*, 13 F.3d 1487, 1489 (11th Cir. 1994) (*Miranda* warnings must precede any "custodial interrogation.>").

"Custodial interrogation" occurs whenever law enforcement officers question a person after taking that person into custody or otherwise significantly deprive a person of freedom of action. *Rogers*, 659 F.3d at 77 (citing *Miranda*, 384 U.S. at 444).

Even if a person has not been formally arrested, advice of *Miranda* rights is required if there is a restraint on freedom of movement "of the degree associated with a formal arrest." *Minnesota v. Murphy*, 465 U.S. 420, 430 (1984). The test is objective; "[t]he only relevant inquiry is how a reasonable man in the

suspect's position would have understood his situation.” *Berkemer v. McCarty*, 468 U.S. 420, 442 (1984). “[I]n order for a court to conclude that a suspect is in custody, it must be evident that, under the totality of the circumstances, a reasonable man in the suspect's position would feel a restraint on his freedom of movement fairly characterized as that ‘degree associated with a formal arrest’ to such extent that he would not feel free to leave.” *Id.* (citations omitted). “[U]nder the objective standard, the reasonable person from whose perspective ‘custody’ is defined is a reasonable innocent person.” *United States v. Moya*, 74 F.3d 1117, 1119 (11th Cir. 1996) (emphasis added). Due to the differences in military and civilian life, a member of the military may be deemed to be in custody for *Miranda* purposes when subject to a direct order to report to a certain place or to make a statement. *See, e.g., Rogers*, 659 F.3d 79.

The Defendant concedes that the Defendant “was not ‘in custody’ for purposes of *Miranda*.” Dkt. 35 at 4. Despite that concession, the Defendant argues, to the contrary, that *Miranda* warnings were required because the Defendant was in custody for *Miranda* purposes, in the military context. *Id.* at 10 (“The Court should suppress Defendant’s statements because Rear Admiral M.J. and Captain C.G. failed to give a rights warning as required by the Fifth Amendment. Interpreting the Fifth Amendment, *Miranda* requires a rights warning when there is a custodial interrogation.”).

The facts show that the Defendant was not in custody under any formulation; he was never ordered to appear anywhere or to make a statement, and the Defendant does not allege that he was. With the exception of his brief conversation with Captain C.G. at the airport when he was departing GTMO all of the Defendant's statements from January 12, 2015 and thereafter were made to Rear Admiral M.J. and Captain C.G. over the telephone. The Defendant argues that this fact is of no moment (Dkt. 35 at 9) but it is relevant when considered as part of the totality of the circumstances, as is the fact that the Defendant initiated most of those phone calls of his own volition for the purpose of volunteering information to Rear Admiral M.J. and Captain C.G. The one phone call that the Defendant was directed to initiate was the January 13 call to Rear Admiral M.J., but that directive was not a direct order, and the Defendant was not ordered to make any incriminating statements on the phone call. None of the circumstances suggest that the Defendant did not feel free to terminate the encounters with Rear Admiral M.J. or Captain C.G.¹ He was never in custody for *Miranda* purposes.

¹ To the extent that the Defendant argues that the directive from Rear Admiral M.J. to call him was effectively an order to the Defendant to appear and make a statement, the record shows that the Defendant feels comfortable ignoring such directives. Rear Admiral M.J. also directed—not ordered—the Defendant to provide the information he gave to her to NCIS, which the Defendant did not do. Clearly the “subtle pressures” and “subtle coercions” of the military hierarchy did not overbear the Defendant's will with regard to notifying NCIS.

The Defendant acknowledges that other courts have found that servicemembers in situations similar to, if not more apparently custodial than, the Defendant's situation were found not to be custodial. Dkt. 35 at 12. In *United States v. Santiago*, a Navy Lieutenant was questioned by NCIS investigators, during what the Lieutenant understood to be an administrative inquiry. 966 F. Supp. 2d 247, 261 (S.D.N.Y. 2013). The Lieutenant was told the interview was voluntary, remained armed during the interview, was not restrained in any way, was not accused of wrongdoing, and remained calm during the interview. *Id.* The court found that the circumstances of the interview were non-custodial, for *Miranda* purposes. *Id.* at 264.

The Defendant argues that the *Santiago* court “fails to adequately recognize the ‘subtle pressures’ or ‘subtle coercion’ in the military context that undermines the voluntariness of statements made by inferior officers when questioned by superiors.” Dkt. 35 at 12. This is not so; the *Santiago* court directly addressed that argument:

[I]n a classically hierarchical institution like the military, where a subordinate has no choice but to obey the lawful order of a superior, a “reasonable person” in Defendant's position might well feel “coerced” to participate in what is described to him as a “voluntary” interview with a superior officer—even more “coerced” than a civilian feels when a police officer stops him and asks him questions.

Santiago, 966 F. Supp. 2d at 261. According to the *Santiago* court, the fact that

a servicemember may feel an additional amount of “coercion” when interviewed by a superior officer, “does not mean, however, that every time a superior questions a subordinate in the military, the subordinate is ‘in custody’ within the meaning of *Miranda*.” *Id.* at 262. The court followed this with “*Rogers* does not go so far,” referencing *United States v. Rogers*, 659 F.3d 74 (1st Cir. 2011), cited by the Defendant for the proposition that the “subtle coercion” and “subtle pressure” present in the military context renders any questioning by a superior officer to be custodial and involuntary. Dkt. 35 at 12. As the *Santiago* court recognized, this is not the law.

In *Rogers*, the First Circuit reiterated that *Miranda* applies in the military context, and that the question of whether a servicemember is in custody turns on “whether a reasonable person in the circumstances would have felt at liberty to terminate the interrogation and leave.” 659 F.3d at 78 (internal citation and quotation marks omitted). The court found in that case that a non-commissioned officer was in custody for *Miranda* purposes where he was ordered by a superior officer to return home, where he found military police executing a search warrant and questioning his pregnant wife. *Id.* The critical fact in finding that he was in custody was “[n]ot only was he under a military order to be there at the time, but a reasonable person could not have doubted that the commanding officer had been aware of what was ahead and was

purposely ordering his subordinate into the company” of military law enforcement. *Id.* The court stated that “this order reasonably carried at least (and probably more than) what the . . . ‘subtle coercion,’ . . . and ‘subtle pressures,’ to speak with the representatives of authority. *Id.* at 78 (internal citations omitted, emphasis added). By contrast in this case, the Defendant was not ordered to appear anywhere or to make any statements.

However, even if the Defendant was ordered to appear, or if Rear Admiral M.J.’s directive to the Defendant to call her was construed as an order, this would not necessitate a finding that he was in custody. In *United States v. Baird*, 851 F.2d 376, 380 (D.C. Cir. 1988), the court found an interview to be non-custodial based on a totality of the circumstances, despite the fact that the fact that the servicemember was ordered to appear for the interview.

The *Santiago* court also recognized that *United States v. Shafer*, 384 F. Supp. 486, 489 (N.D. Ohio 1974), also relied upon by the Defendant, does not hold “that every interview of a military subordinate by a superior officer is custodial in nature. In fact, *Shafer* plainly holds to the contrary.” *Santiago*, 966 F. Supp. 2d at 262, *see* Dkt. 35 at 10-11. In *Shafer*, the court stated that “[i]t has been held that ‘custody’ over military personnel occurs when there has been some assumption of control over their movements. . . . This ‘control’ may take many forms, such as ordering a person to appear at a specified time and place

for the purpose of making a statement.” 384 F. Supp. at 488 (internal citations omitted). In light of that principle, the court held that servicemembers were in custody when ordered to report to a specific location with the express intention that they make a statement. *Id.*

The facts show that the Defendant’s statements to Rear Admiral M.J. and Captain C.G. were volunteered by the Defendant in phone calls that he initiated. He was never ordered to appear anywhere or to make statements. No Navy personnel assumed control over his movements or made him feel that he was not free to terminate the encounter. The Defendant was not in custody for *Miranda* purposes at any time, and therefore was not entitled to *Miranda* warnings. None of his statements were obtained in violation of *Miranda*.²

3. The Defendant’s Statements Were Voluntary.

A statement obtained not in violation of Article 31 or *Miranda* may nevertheless violate a defendant’s Fifth Amendment rights if it was not voluntarily given. *Schneckloth v. Bustamonte*, 412 U.S. 218, 225 (1973). The Defendant argues that his statements made to Rear Admiral M.J. and Captain C.G. beginning on January 12, 2015 were involuntary under the Fifth Amendment. Dkt. 35 at 5-6.

² Although not argued by the Defendant, even if he was “in custody” for *Miranda* purposes, the Defendant was not subject to “interrogation” for *Miranda* purposes, just as he was not being questioned for Article 31 purposes.

In determining whether a defendant's will was overborne in a particular case, the Court has assessed the totality of all the surrounding circumstances—both the characteristics of the accused and the details of the interrogation. Some of the factors taken into account have included the youth of the accused, lack of education, low intelligence, the lack of any advice to the accused of his constitutional rights, the length of detention, the repeated and prolonged nature of the questioning, and the use of physical punishment such as the deprivation of food or sleep. *Schneckloth*, 412 U.S. at 226 (internal citations omitted).

The Defendant's statements were voluntarily given, viewing the totality of the circumstances. The Defendant is a highly-educated and high-ranking officer in the United States Navy who, at the time of these statements, was charged with the command of a Naval Air Station located in a country with whom the United States had, at best, complicated diplomatic relations. In that capacity he was responsible for the lives of 5,000 military and non-military individuals. As commanding officer of GTMO, his responsibility was "absolute" and his authority was "commensurate with his responsibility." Dkt. 1 at ¶ 5(b); U.S. Navy Regulation Ch. 8, § 1, art 0802 (codified at 32 C.F.R. § 700.802). Furthermore, as commander, the Defendant was required to "exercise leadership through personal example, moral responsibility, and judicious attention to the welfare of persons under [his] control or supervision."

Id. Admiral M.J. considered the Defendant one of her “higher rated installation Commanders” who has “historically fully informed [her] relative to any matters requiring [her] attention.” Ex. B at 5. Simply put, the Defendant was not the type of person whose will could be easily overborne.

The Defendant’s statements were offered voluntarily, over the phone in relatively brief phone calls that were initiated by the Defendant, not because of direct questioning, let alone “interrogation” (in the common sense of the word). There was no detention, no questioning intended to elicit incriminating information, and no physical punishment. All the Defendant had to do to terminate the encounter was hang up the phone, or not make the call at all.

The Defendant’s sole basis for his argument that his statements were involuntary are the “inherent ‘subtle pressures’ and ‘subtle coercion’ of [the] military relationship” between the Defendant and his superior officers: Rear Admiral M.J. and Captain C.G. Dkt. 35 at 7. However, the Defendant does not provide a single case finding that the relationship between a superior and inferior officer alone can render a statement involuntary. *See id.* The only two cases cited by the Defendant in support of this proposition, *Rogers* and *Shafer*, found only that the servicemembers in question were in custody for *Miranda* purposes, not that their statements were involuntary. *See* Dkt 35 at 10-11; *Rogers*, 659 F.3d at 77; *Shafer*, 384 F. Supp. at 490. To the contrary, federal

courts have routinely found statements made by servicemembers to their superior officers to be voluntary. *See, e.g., Sassen v. Spencer*, 879 F.3d 354, 365 (1st Cir. 2018) (finding statements made by a Chief Petty Officer to a Lieutenant to be voluntarily made).

The Defendant does not cite any cases to support his position on the voluntariness of his statement. In *United States v. Armstrong*, cited by the Defendant, the U.S. Court of Military Appeals addressed the question of “the admissibility in evidence of a blood specimen extracted from the appellant at a military hospital soon after the automobile accident which gave rise to the charge of involuntary manslaughter.” 9 M.J. 374, 375 (C.M.A. 1980), see Dkt. 35 at 7. The court held that Article 31(b) does not “require that a warning be given before the investigator obtained from a suspect evidence which would not constitute a communication by that suspect.” *Armstrong*, 9 M.J. at 378. In discussing the statutory purpose of Article 31, the court noted that at the time it was passed, it provided unprecedented protections, but Congress nevertheless deemed Article 31 “necessary because of subtle pressures which existed in military society.” *Id.*

The court did not hold, as suggested by the defense, that the “subtle pressures” existing in the interaction between a high-ranking Navy officer and his commanding officer rendered any statement per se involuntary.

Furthermore, although the Defendant is correct that Rear Admiral is one of the highest ranks in the Navy, but it is only one rank above the Defendant's rank, Captain. The Defendant and Captain C.G. shared a rank, though Captain C.G. served as Chief of Staff to Rear Admiral M.J. This is not a situation, however, where a low-ranking enlisted person is being questioned by a senior commissioned officer.

The Defendant argues that Rear Admiral M.J.'s ability—and Captain C.G.'s ability, as a representative of Rear Admiral M.J.—to arrest or confine the Defendant weighs against the voluntariness of the statements, but this ignores that no such threats were made and there is no evidence in the record that such threats were perceived by the Defendant. Dkt. 35 at 9. Furthermore, the Defendant claims that Article 9 could have been invoked if he refused to answer questions, but as described above, the statements made by the Defendant were volunteered, not the product of questioning by Rear Admiral M.J. or Captain C.G., and no such threats to invoke Article 9 were made.

Viewing the totality of the circumstances, the Defendant's statements were voluntarily made.

IX. OPPOSITION TO MOTION IN LIMINE 1 [DKT. 36]

The Defendant moves in limine to exclude certain evidence and

arguments. These motions should be rejected as they are without merit.³

A. **C.D. Can Properly Provide Lay Witness Testimony that the Blood on the Paper Towel He Found in the Area of the Defendant's Personal Boat Dock, which was Later Confirmed to Contain Tur's DNA, Appeared to Have Been Used for Cleaning Blood Up and Not From Being Pressed Against a Wound.**

As discussed above, and alleged in the Indictment (Dkt. 1 at ¶ 48), at approximately 1:00 p.m. on Sunday January 11, 2015, persons still searching for Tur, unaware that his body had been recovered in Guantanamo Bay a few hours earlier, discovered a paper towel with a reddish-brown stain on it near the base of the pier in Nettleton's backyard. DNA testing later confirmed that the bloodstain on the towel was a match for Tur's DNA. Dkt. 1 at ¶ 48.⁴ One of the persons who initially located the towel, C.D., explained to investigators in

³ The Defendant moved in limine to exclude the following three categories of information that the Government does not intend to introduce in its case-in-chief: (1) evidence or comments that the Defendant had an affair with K.B. based on information provided in discovery, such as text messages between them; (2) evidence that the Defendant's son destroyed or attempted to destroy the cell phone that he used to communicate with the Defendant and others on the night of January 9, 2015, and in the days thereafter when Tur was missing and then discovered dead; and (3) that when the Defendant spoke with Captain D.D. on January 10, 2015, while Tur was missing but not yet found dead, the Defendant spoke about Tur as though he were already dead. Dkt. 36 at 4-5. Accordingly, the Government does not address those arguments herein. In the event the Government decides to attempt to introduce this evidence, such as in response to answers provided by the Defendant if he testifies, the Government will notify the Court and the Defense.

⁴ Later in the day, personnel from CID and NCIS arrived to recover the paper towel. Dkt. 1 at ¶ 48. Nettleton was nearby when one of the Navy personnel recovering the item stated that it appeared to have blood on it, and Nettleton responded, "That's probably nothing." *Id.*

an interview that when he first saw the towel, he observed what appeared to be blood on it, turned it over with a stick, and then saw that the towel was saturated more heavily on the bottom. C.D., who is a 22-year veteran of the Department of Defense Fire Service and has advanced training as an emergency medical technician (“EMT”), told investigators that the saturation appeared to be consistent with coming from cleaning up blood and not from it being pressed against a wound.

The Defendant argues that C.D.’s description of the appearance of the bloodstain should be excluded from the trial because it is a “speculative and unreliable” lay opinion, C.D. “did not observe how the blood got onto the paper towel,” and C.D. is not testifying as an expert witness. Dkt. 36 at 1-2. The Defendant’s arguments should be rejected for the reasons discussed below.

1. Legal Standard

Under Federal Rule of Evidence 602, a “witness may testify to a matter only if evidence is introduced sufficient to support a finding that the witness has personal knowledge of the matter. Evidence to prove personal knowledge may consist of the witness’s own testimony. This rule does not apply to a witness’s expert testimony [admitted] under Rule 703.” In applying Rule 602, courts have allowed witnesses who are not qualified as experts to testify about common occurrences that the witnesses have observed.

For example, a police officer was able to testify that “‘a lot of the residents’ in the Hickory public housing project sell crack to supplement their incomes” because he was familiar with drug dealing in a neighborhood housing project. *United States v. Smith*, 451 F.3d 209, 223 (4th Cir. 2006). Likewise, in applying Rule 602, courts have held that it does not matter that not every part of a witness’s testimony on a subject is based solely on direct sensory perception. *See, e.g., United States v. Neal*, 36 F.3d 1190, 1206 (1st Cir. 1994) (bank employee could testify about bank’s status as an FDIC insured institution and its nexus to interstate commerce, even when that knowledge was derived in part from reviewing records, because personal knowledge under Rule 602 “can include inferences and opinions, so long as they are grounded in personal observation and experience.”) (internal quotation marks and citations omitted).

Additionally, under Rule 701, the opinion of a lay witness is admissible if it is: (a) rationally based on the witness’s perception; (b) helpful to clearly understanding the witness’s testimony or to determining a fact in issue; and (c) not based on scientific, technical, or other specialized knowledge within the scope of Rule 702” regarding the qualifications for expert witnesses. Even if a lay witness possesses specialized knowledge, “Rule 701 does not prohibit lay witnesses from testifying based on particularized knowledge gained from their own personal experiences.” *United States v. Hill*, 643 F.3d 807, 841 (11th Cir.

2011) (in prosecution for mortgage fraud and money laundering, proper for employees of victim financial institutions to testify as lay witnesses about whether their financial institutions would have granted loans if not for fraudulent representations because it was based on the witnesses' personal experiences as officers of financial institutions with knowledge of their companies' policies and the specific transactions at issue); *see also United States v. Wilson*, 605 F.3d 985, 1025 (D.C. Cir. 2010) ("Unlike experts, lay witnesses must base their testimony on their experiential perception and not on scientific, technical, or other specialized knowledge within the scope of Rule 702. This requirement ensures that lay testimony is the product of reasoning processes familiar to the average person in everyday life. Moreover, it avoids the risk that the reliability requirements set forth in Rule 702 will be evaded through the simple expedient of proffering an expert in lay witness clothing.") (citations and internal quotation marks omitted); *United States v. Hassan*, 742 F.3d 104, 135-36 (4th Cir. 2014) (witnesses can draw inferences about conversations in which they participated because under Rule 701, a lay witness may draw inferences that are "rationally based on the witness's perception," if those inferences require no specialized expertise); *United States v. Aguilar-Acevedo*, 488 F. App'x 243, 244 (9th Cir. 2012) (in matter involving charges of assault on a federal officer, the trial court "did not err in permitting lay witnesses to establish bodily

injuries because the testimony was based on the witnesses' personal observations and recollection of concrete facts, and it did not require specialized or technical knowledge to establish that a person was injured by rocks hitting his head."); *Barnes v. District of Columbia*, 924 F. Supp. 2d 74, 83 (D.D.C. 2013) ("Lay opinion testimony is admissible if the specialized knowledge at issue was gained through experience rather than through scientific or technical training, so long as the witness testified based solely on personal experience with the case at issue.") (internal quotation marks and citations removed).

2. C.D.'s Testimony About the Appearance of the Bloodstain on the Paper Towel is Admissible and is Not Improper Expert Testimony.

C.D. is expected to testify that when the towel was first discovered, the heavy saturation of the blood on the bottom of the paper towel appeared to him to be consistent with the towel being used to clean up blood rather than being pressed against a wound. This testimony is relevant to establish that the towel may have been used to aid in the charged obstruction and concealment offenses. It is factual testimony that is based on his personal observations, as supplemented by his normal experiences as a veteran of the DOD Fire Service and as a trained EMT, and as such it is well within the bounds of Rule 602.

To the extent that C.D.'s testimony is a lay opinion instead of fact

testimony based on C.D.’s personal knowledge—which the Government does not concede it is—it would also be properly admitted under Rule 701 as it is “based on particularized knowledge gained from [C.D.’s] own personal experiences.” *Hill*, 643 F.3d at 841. Describing the bloodstain, based on personal observations, is no different than a witness explaining that a car appeared to be speeding, or that a person appeared to be drunk, which are all types of statements permissible as lay opinions. *United States v. Marshall*, 173 F.3d 1312, 1315 (11th Cir. 1999) (holding that testimony by officer that drugs had to come from the defendants was inadmissible because under Rule 701, “the opinion of a lay witness on a matter is admissible only if it is based on first-hand knowledge or observation—for example, a witness’ opinion that a person with whom he had spoken was drunk, or that a car he observed was traveling in excess of a certain speed.”).

Although “Rule 701 forbids the admission of expert testimony dressed in lay witness clothing,” Rule 701 “does not interdict all inference drawing by lay witnesses.” *United States v. Perkins*, 470 F.3d 150, 156 (4th Cir. 2006) (quoting *United States v. Santos*, 201 F.3d 953, 963 (7th Cir. 2000)). Such lay inferential opinion testimony is helpful to a jury because often a witness may be able to describe some details that produced the opinion, but not all, and the lay opinion is helpful in such a case because it “fills gaps that would be left by an incomplete

description of the witness's perceptions." *See* Helpful to Understanding Testimony Determining a Fact, 29 Fed. Prac. & Proc. Evid. § 6255 (2d ed.) (giving example that a lay witness can opine that a person was angry because, although the witness can describe that he observed the person with a furrowed brow and a frown, the opinion that the person was angry provides more clarity than does describing the perceptions that made the witness believe the person was angry as opposed to experiencing some other emotion). Accordingly, the type of factual description C.D. can provide for the jury is both helpful and properly admissible under both Rules 602 and 701.

B. Evidence Concerning the Results of the Use of the Forensic Tool "Bluestar" to Search for the Presumptive Presence of Blood in the Defendant's Residence and on His Private Boat Dock is Properly Admissible Under the *Daubert* Standard Because Bluestar is Scientifically Reliable and DNA Testing Confirmed that Tur's Blood Was Recovered from Both Locations.

The Defendant has moved to exclude evidence and testimony concerning investigators' use of the forensic tool known as "Bluestar" to test for the presence of blood at the Defendant's residence and on the railings of his private boat dock outside his residence because, in the Defendant's view, "such testimony is based solely on the purported discovery of blood through the use of Bluestar" and does not meet the standard for the admission of scientific evidence established in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 (1993). Dkt. 36 at 2-3.

The Defendant's arguments are based on a misreading of both the law and the facts concerning the forensic evidence recovered from both the residence and the area of the boat dock. Bluestar is a reliable presumptive test for the presence of blood, and evidence concerning Bluestar—such as testimony regarding its use and photographs that show where the application of Bluestar yielded positive results for the presumptive presence of blood—meets the standards for admission established in *Daubert*. In any event, the Defendant incorrectly asserts that there was no confirmation of the Bluestar results, even though DNA testing confirmed the presence of Tur's blood and/or DNA in locations where Bluestar testing had shown the presumptive presence of blood in the residence and in the area of the boat dock. Accordingly, the Defendant's arguments should be rejected.

1. Factual Background

This background information is a summary of information provided in the Indictment and in reports and other materials provided in discovery, as well as expert notices provided by the Government regarding expected testimony by the Government's DNA experts.

Beginning on or about January 20, 2015, NCIS agents conducted several searches of the Defendant's home and the surrounding curtilage, including his private boat dock reached by way of a series of stairs and a walkway that begins

near the front of his residence. During the initial search that began on or about January 20, agents observed, and took swabs of, what appeared to be visible bloodstains at several locations on the first floor of the Defendant's residence, notably in the bar room and a sitting room next to the bar room. Samples of the suspected visible bloodstain swabs were sent for serology and DNA testing, and where results could be obtained from laboratory testing, they were all confirmed to be blood and/or to contain DNA that matched Tur's DNA profile. None of the Defendant's DNA was found on these swabs of the visible bloodstains.⁵

Additionally, during the initial searches that began on or about January 20, after locating and swabbing the visible suspected bloodstains, NCIS agents also used Bluestar to search for the potential presence of blood in the sitting room, the bar room, elsewhere inside and outside the residence, and on the walkway and railing leading to the boat dock. As discussed further herein, Bluestar is a patented latent bloodstain reagent that reacts to certain organic compounds, such as blood. Forensic examiners are trained to apply it to

⁵ A visible suspected bloodstain was found on sheets on the second floor of the residence that later was confirmed through serology testing to be blood and to contain a DNA profile belonging to an unknown female that was not Tur's spouse, based on a comparison to a DNA profile obtained from her by investigators. A DNA sample was never obtained from the Defendant's female family members, so it is unknown whether the blood on the sheets belonged to them.

surfaces to test presumptively for the presence of blood.⁶ After Bluestar is applied to a surface, an investigator examines the surface using special lighting, which reveals a luminescent glow that can be photographed in locations where the Bluestar reacted to blood or other organic compounds. In this case, Bluestar was applied to several locations in the sitting room and bar room. That application revealed positive luminescence results in several locations, including in locations where visible bloodstain swabs also were taken which were later found to contain blood and/or Tur's DNA.⁷ Forensic investigators photographed the positive luminescence. Bluestar was also applied to some locations in the bar room, sitting room, elsewhere inside the residence, and on the exterior of the residence (such as an exterior door and steps leading out), where there were no visible bloodstains and no swabs were taken, but the Bluestar nevertheless revealed positive luminescence results.

During the initial searches that began on or about January 20, Bluestar

⁶ Bluestar can also reveal a positive luminescence when applied to surfaces that have certain other organic compounds on them, such as compounds found in bleach and other household cleaners. Attached as Exhibit C are the instructions that come with Bluestar regarding its use and reactions. *See also Bluestar® Forensic Latent Blood Reagent*, available at <https://www.bluestar-forensic.com/gb/bluestar.php> (last visited July 30, 2019).

⁷ As an example of these types of results for the Court to review, attached as Exhibit D is a comparison of color photographs of positive Bluestar testing results in two locations in the bar room with photographs of visible bloodstains that were later found to contain Tur's DNA.

was also used on the Defendant's private boat dock area.⁸ Several locations on the railing and steps of a walkway leading to the boat dock had positive luminescence results, and swabs were taken from those locations. Some of these swabs could not be determined to contain blood or DNA, but one of the swabs (D5) was found to contain a partial DNA profile that was consistent with Tur's DNA.⁹

Beginning on or about March 7, 2015, NCIS agents returned to the Defendant's home to conduct additional Bluestar testing on the Defendant's boat dock and on his private boat. Application of Bluestar to some areas of the dock and boat resulted in positive results, but use of another forensic tool, Hexagon OBTI, which alerts to the presence of human hemoglobin, revealed

⁸ The bloody paper towel found on January 11, 2015, and later discovered to be a match for Tur's DNA, discussed above, was located on the ground near the steps and walkway leading to the boat dock.

⁹ The Defendant's motion appears to contain a misreading of the evidence provided in discovery when he asserts that "the samples from the railings were not subsequently confirmed to contain blood via a more reliable testing method," Dkt. 36 at 2-3, given that swab D5, taken of suspected blood on the dock area—which was discovered through the application of Bluestar—resulted in the discovery of a partial DNA profile consistent with Tur's DNA profile.

For the Court's reference, attached as Exhibit E are excerpts from a forensic report provided in discovery that contains photographs of the area of the boat dock and the walkway leading to it; the bloody paper towel found near the dock's walkway and stairs that matched Tur's DNA; and the areas of the walkway's railing and steps where Bluestar revealed a positive luminescence, including where the swab (D5) was lifted that had a partial DNA profile consistent with Tur's DNA.

negative results for blood, so no swabs were taken and no further testing was conducted.¹⁰

2. Legal Standard

When considering the admissibility of scientific evidence in the context of the proposed testimony of an expert witness, pursuant to Rule 702, a trial court has a “critical ‘gatekeeping’ function concerning the admissibility of expert scientific [and technical] evidence.” *United States v. Abreu*, 406 F.3d 1304, 1305–07 (11th Cir. 2005) (upholding the admissibility of fingerprint evidence under the reliability standards of *Daubert*) (quoting *United States v. Frazier*, 387 F.3d 1244, 1260 (11th Cir. 2004)). To assess “reliability,” a trial court “considers a number of factors, including those listed by the Supreme Court in *Daubert*: (1) whether the expert’s theory can be and has been tested; (2) whether the theory has been subjected to peer review and publication; (3) the known or potential rate of error of the particular scientific technique; and (4) whether the technique is generally accepted in the scientific community.” *Id.* at 1306-07 (citing *Frazier*, 387 F.3d at 1262). “The *Daubert* factors are only illustrative and may not all apply in every case.” *Id.* at 1307. “The district

¹⁰ Consistent with the results of the testing conducted on or about March 7, 2015, the Government is not intending to admit evidence or arguments indicating that the results of the testing on that date revealed the presence of any blood. To the contrary, if the Government admits evidence of the search and testing on March 7 at all, it will do so in order to explain that NCIS attempted to search for more blood but did not recover any evidence of it on that date.

court has wide latitude in deciding how to determine reliability.” *Id.* (citing *Kumho Tire Co. v. Carmichael*, 526 U.S. 137, 142 (1999) (applying *Daubert* standards to the admissibility of expert opinions about non-scientific matters)).

3. Evidence Concerning the Results of Bluestar Testing is Reliable and Admissible.

As an initial matter, it should be noted that the Government does not intend to admit expert testimony concerning the Bluestar testing conducted at the Defendant’s residence and in the dock area.¹¹ Indeed, the Government does not intend to admit any opinion testimony whatsoever concerning the Bluestar testing or the results of it. Instead, the Government intends to have the NCIS crime scene agents who conducted the search of the residence and boat dock area testify about what Bluestar is and why they used it based on their training and experience (*i.e.* they used it to search for the potential presence of blood because Bluestar reacts to organic compounds, like blood, as well as bleach and other organic compounds in cleaning products), how and where they applied Bluestar during the search, and that the Bluestar yielded luminescent results in some locations as documented in photographs that will be introduced

¹¹ The Government will admit expert testimony of DNA examiners concerning the match to Tur’s DNA profile of the DNA profile obtained from the swabs NCIS agents took of the visible bloodstains in the residence and the suspected bloodstain taken from the boat dock area. These experts will also testify about the serology testing that confirmed the presence of blood in many of the swabs. The Defendant has not objected to the admissibility of this expert testimony.

in evidence.¹² Such testimony is analogous to crime scene technicians testifying about numerous different methods and techniques of recovering evidence, such as an agent explaining how she swabbed a gun with magnetic powder to lift a potential latent fingerprint that she then sent to a lab for fingerprint comparison. The NCIS crime scene agents will not opine that the luminescence means that the Bluestar detected blood, bleach, cleaning products, or anything else in those locations.

Accordingly, the critical question regarding the admissibility of the non-expert testimony and evidence concerning the Bluestar testing is not whether it is admissible through expert testimony as a reliable scientific method and opinion under the strictures of *Daubert* and Rule 702, but instead whether the use of Bluestar and its results is relevant under Rule 401; that is whether it has a “tendency to make a fact more or less probable than it would be without the evidence.” FED. R. EVID. 401. Here, the Bluestar evidence is relevant because it shows locations where the luminescent results revealed the possible location of organic compounds—such as blood or cleaning products like bleach.

The presence of luminescent stains consistent with blood or cleaning products like bleach—and the confirmation that swabs of the visible blood

¹² These NCIS crime scene agents will also testify about other evidence from the search, such as that they discovered the visible suspected bloodstains and took swabs of the stains that were sent for serology and DNA testing, took photographs of the residence and boat dock area, and seized certain items.

matched to Tur's DNA profile—establishes that Tur was at Nettleton's house after the argument at the Officer's Club and that a serious altercation occurred there, which caused Tur to bleed. These are the events that the Defendant concealed, made false statements regarding, and sought to prevent others from discovering through his obstruction as charged in the Indictment. Dkt. 1 at ¶¶ 60-77.¹³

To the extent that the Court believes that it must test the reliability of Bluestar as a scientific method under the *Daubert* standard, there is ample evidence that Bluestar is reliable as a presumptive test for the presence of blood, even if it also can have positive luminescence for some other organic compounds, such as bleach or other cleaning products. While the Government has not found a federal case directly ruling on the admissibility of Bluestar, which is a proprietary product, there are numerous opinions admitting other similar presumptive tests for the presence of blood and similar biological

¹³ To the extent the NCIS crime scene agents' testimony concerning the use of Bluestar contains any opinion at all, it would be admissible as a lay opinion under Rule 701 given that the agents would be testifying based on their personal knowledge of the use of the Bluestar during the searches. See *United States v. Variste*, 625 F. App'x 458, 460 (11th Cir. 2015) (admitting lay opinion testimony of Internal Revenue Service agent about indicators of fraud on defendant's tax returns where agent's testimony was based on her personal review of tax returns, her testimony about fraud indicators helped the jury better understand the significance of commonalities in tax returns, testimony was based on summary of documents related to the case, and the jury could have reviewed the documents itself); *Hill*, 643 F.3d at 841 ("Rule 701 does not prohibit lay witnesses from testifying based on particularized knowledge gained from their own personal experiences.").

information. For example, in *United States v. Fell*, No. 5:01-cr-12, 2017 WL 10809984 at *1 (D. Vt. Feb. 8, 2017), the court admitted, pursuant to *Daubert*, the results of a phenolphthalein test that presumptively detects the presence of hidden blood, even while it acknowledged that the test “has a significant drawback” in that “[c]ommon substances which also cause stains including rust, beet juice, horseradish, and animal blood will also test positive,” as long as the evidence was “carefully limited to the presumptive nature of the test.” *See also United States v. Christensen*, No. 17-cr-20037-JES-JEH, 2019 WL 651500, *3 (C.D. Ill. Feb. 15, 2019) (admitting luminol and phenolphthalein tests that presumptively detect the presence of hidden blood); *United States v. Rodriguez*, 581 F.3d 775 (8th Cir. 2009) (admitting an acid-phosphate test that presumptively tests for the presence of semen); *United States v. Lonedog*, 67 F. App’x 543, 548-50 (10th Cir. 2003) (admitting a presumptive test for the presence of urine); *United States v. Diaz*, No. CR 05-0167 WHA, 2006 WL 3512032, *10-11 (N.D. Cal. Dec. 6, 2006) (admitting a cobalt thiocyanate color test that presumptively identifies a substance as cocaine); *United States v. Perez*, No. 7:11-CR-181, 7:11-CR-170, 2012 WL 243232, *3 (W.D. La. Jan. 25, 2012) (admitting a Narcotics Analysis Reagent test that presumptively identifies a certain substance as a drug, holding that “reagents tests are clearly more than ‘unsupported speculation,’ and are sufficiently based on ‘good grounds’ to be

reliable under Rule 702.”).

Scientific tests conducted of Bluestar on multiple occasions have also demonstrated Bluestar’s efficacy when compared with other presumptive tests for the presence of blood (including as confirmed through later DNA testing), the results of which were later published in journals for forensic scientists. *See, e.g.* Ex. F (Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA, J. Forensic Sci., January 2007, Vol. 52, No. 1; Presumptive tests: A substitute for Benzidine in blood samples recognition, Forensic Science International: Genetics Supplement Series 6 (2017); Effect of presumptive tests reagents on human blood confirmatory tests and DNA analysis using real time polymerase chain reaction, Forensic Science International 206 (2011); Use of Bluestar Forensic in Lieu of Luminol at Crime Scenes, Journal of Forensic Identification, 706/56(5) (2006)).¹⁴

According to NCIS Agent Carrie McNamara, one of the agents who processed items from the Defendant’s residence in search of blood, the use of Bluestar as a presumptive test for blood is taught at the Federal Law

¹⁴ Numerous other studies concerning the reliability and efficacy of Bluestar as a presumptive test for blood can be found at: <https://www.bluestar-forensic.com/en/our-products/bluestar-forensic/documentation> (last visited August 14, 2019).

Enforcement Training Center (“FLETC”). Furthermore, the International Association for Identification offers a workshop (which she graduated from) entitled “Using Luminol & Bluestar Forensic for the Identification of Possible Blood Stains at the Crime Scene” at its educational conference.

Furthermore, as discussed above, there is scientific evidence of the efficacy of Bluestar in this very case, as demonstrated by the confirmation through serology and DNA testing of the presence of blood and/or Tur’s DNA at numerous swabbed locations in the bar room and sitting room in the Defendant’s residence, as well as on the boat dock, where Bluestar also had a positive luminescence.

Accordingly, evidence and testimony concerning the use of Bluestar during the search of the Defendant’s residence and boat dock area is properly admissible in this case. The Defendant is entitled to argue that the positive luminescence could also be consistent with the presence of other organic compounds that Bluestar can react to, but such arguments go to the weight, not admissibility, of the evidence. *See Christensen*, 2019 WL 651500, *3 (“Defendant’s arguments about the reliability of the [luminol and phenolphthalein] test results and the appropriate weight to assign them may be presented to the jury at trial, but they do not persuade the Court to exclude the results of the luminol or phenolphthalein tests.”). Indeed, the jury would be

free to conclude that the positive luminescence from the application of Bluestar in some locations inside the residence could be consistent with the presence of a cleaning product like bleach, as well as blood, which would be consistent with the Defendant making efforts to conceal the blood from his fight with Tur.

C. The Government Can Properly Argue that the Defendant's Obstruction, Concealment, Falsification of Records, and False Statements Contributed to an Inability to Determine the Manner of Tur's Death.

The autopsy of Tur conducted by a Navy medical examiner, Dr. Christopher Gordon, determined that the cause of Tur's death was "probable drowning in the setting of ethanol and fluoxetine toxicity," but that his manner of death was undermined.¹⁵ Tur's body was decomposed as a result of floating in water for an extended period. Among other things, the autopsy revealed that Tur had a laceration on his head and that before he died, Tur sustained rib fractures with associated soft tissue damage. The Defendant has moved *in limine* to prevent the Government from "mak[ing] any statements which imply the Defendant killed Tur, or that the result of the Defendant's actions is that we'll never know exactly what happened to Tur." Dkt. 36 at 3. The

¹⁵ Dr. Gordon is currently the Chief of Medical Staff for the 71st Medical Group, Vance Air Force Base, Oklahoma. At the time he performed the autopsy of Christopher Tur, he was the Director of Operations of the Office of the Armed Forces Medical Examiner ("AFME"). The Government has provided notice to the Defense that it intends to call Dr. Gordon to testify as an expert in the field of forensic pathology regarding his opinion about the cause and manner of the death of Tur, as well as to testify as a fact witness about what he observed and did during the autopsy

Defendant argues these comments are not supported by the evidence, are “based entirely on impermissible speculation and conjecture,” and are irrelevant and prejudicial. *Id.* These arguments should be rejected. While the Government does not intend to argue that the Defendant actually murdered Tur, it is entirely proper to argue to the jury that the Defendant’s obstruction, concealment, falsification of records, and false statements contributed to the inability to determine the manner of Tur’s death.

For example, the Defendant concealed from Navy investigators and his superior officers that Tur had come to his house after the party at the Officer’s Club, and that they engaged in a physical altercation there that resulted in injuries to Tur—as evidenced by the testimony and contemporaneous text messages of the Defendant’s daughter and the recovery of Tur’s blood in the house. This concealment prevented investigators from knowing that they should focus their search for Tur on the area of Deer Point and the surrounding waters when Tur was still missing, which may have contributed to his body not being found until it had floated in water and decomposed for almost two days.

Indeed, the false statements and obstruction the Defendant engaged in beginning on the morning of Saturday, January 10, 2015 actually resulted in investigators and others focusing the search for Tur in the *opposite* direction from the Defendant’s residence at the tip of Deer Point, such as when the Defendant

told Command Duty Officer A.T that Tur was last seen at the Officer's Club, without mentioning the altercation afterwards at the Defendant's residence, when he instructed him to have the GTMO Security Department start searching for Tur. Indict. ¶ 30-32.

The concealment, obstruction, and false statements also delayed investigators for substantial periods of time from finding witnesses to relevant events, such as the argument at the Officer's Club, and prevented them from locating and preserving evidence at the scene of the physical altercation until nine days after Tur's death, during which time some of the evidence could have been cleaned up or affected by the elements.

Simply put, investigators were unable to determine how the physical altercation with Tur began, how Tur was injured such that he bled in the Defendant's residence, how Tur suffered serious rib fractures before his death, how the altercation ended, how Tur left the Defendant's residence, how or why Tur's DNA was found on the walkway of the boat dock or on a bloody paper towel next to it, or how Tur ended up in the water such that he drowned. The Defendant's obstruction, concealment, falsification of records, and false statements contributed to the existence of these open questions about the manner of Tur's death. Their inability to resolve these open questions is relevant as evidence to explain why the defendant would have sought to take

obstructive acts in the first place.

Regardless of whether the Defendant murdered Tur or not by causing him to enter the water in which he drowned, the Defendant was facing, at the very least, potential investigation and court martial for conduct unbecoming an officer (based on his public drunkenness at the Officer's Club), adultery (based on his affair with Tur's spouse, of which he had been accused in public before numerous witnesses by Tur), and assault (for his altercation with Tur inside his residence and the injuries caused in the altercation). As such, even assuming he was not responsible for Tur entering the water, and thus did not cause Tur to die by drowning, the Defendant still had a motive to commit the charged obstructive offenses. The fact that the Defendant's obstructive acts were effective in hindering the investigation into the circumstances of Tur's disappearance makes it more probable that the Defendant engaged in those acts with the specific intent to obstruct the investigations, an element of certain of the charged crimes. *See supra* at 15 (discussing the elements of the obstruction of justice counts, including the element in Section 1512(b)(3) that the defendant acted "with the intent to hinder, delay or prevent the communication of information to a federal official," and in Section 1512(c)(2) that the defendant "acted corruptly, *i.e.*, with an improper purpose and to engage in conduct knowingly and dishonestly with the specific intent to subvert, impede or

obstruct the proceeding.”).

Courts have permitted prosecutors to make the point in argument and through evidence that there was a motive to commit obstruction, concealment, and related crimes based on uncharged crimes, and that these acts had their intended effect by hindering investigations. For example, in *United States v. Fumo*, 2009 WL 1688482 (E.D. Pa. June 17, 2009), the defendants were charged with several counts of fraud and one count of obstruction/conspiracy to obstruct. In closing argument, government counsel mentioned some of the possible motivations that the defendant could have had for conspiring to commit fraud, including saying, “Maybe Mr. Carter in some way, there’s some kind of bribery or extortion going on – if I give you the yacht, I want to see some more grants. Maybe –.” *Id.* at *44. The defendant argued that this was prejudicial because he was not charged with bribery or extortion. The court rejected the defendant’s argument that this reference unduly prejudiced the defendant, noting that “[t]he prosecutor simply indicated that the jury could ponder any number of reasons for the dealings between Carter and Fumo, offering a few suggestions such as deceit, bribery, extortion At no point did the prosecutor accuse or ask the jury to find Fumo guilty of any uncharged crime.” *Id.* at *45.

Similarly, in *United States v. Lalley*, 2010 WL 3946659 (D.N.J. Oct. 5,

2010), the defendant, a police officer who was being investigated for stealing money and narcotics from drug dealers, disclosed during the investigation that he had a relationship with a male in the past and then subsequently instructed the victim not to reveal to investigators that they had a relationship while the victim was a minor. The defendant was indicted on two counts of obstruction, in violation of 18 U.S.C. §1512. The defendant moved to strike as surplusage a section of the indictment titled “Allegations Under Investigation” for being irrelevant and prejudicial. In response, the government argued that the details were relevant and material to the indictment because the indictment must contain the elements of the offense charged and to establish obstruction, the government had to show that the defendant was being investigated by a grand jury and law enforcement authorities. The court struck the term “victim” from the indictment, but kept the underlying allegations regarding the uncharged statutory rape. The court noted that “it is clear that the details described as ‘Allegations Under Investigation,’ are relevant and material to the government’s ability to establish the obstruction charges against defendant.” 2010 WL 3946659 at *4. The court held, “While the underlying alleged crimes are not essential elements for the obstruction of justice charges currently before this Court, the nature of the allegations brought before the Grand Jury for its consideration are nevertheless relevant to the overall scheme of the obstruction

charges in the current indictment.” *Id.*

A number of other courts have permitted arguments concerning the underlying uncharged crimes a defendant was attempting to obstruct, even where these underlying crimes were quite prejudicial. *See, e.g., United States v. Schnurstein*, 977 F.2d 449, 454 (8th Cir. 1992) (holding that the prosecutor’s arguments concerning underlying uncharged drug crimes were permissible where the defendant was charged with obstruction of justice for attempting to intimidate a witness to prevent him from providing information on the defendant’s drug activity, reasoning that “The comments were proper in light of the fact that the defendant intended to hinder, delay or prevent [the witness] from disclosing information related to drug offenses.”); *United States v. Cummins*, 969 F.2d 223, 227 (6th Cir. 1992) (prosecutor’s arguments, where the defendant was charged with suborning perjury during a drug investigation, that referred to the defendant’s “association with [the original target’s] drug dealings” were not in error because “as the jury was aware, the case against [the defendant] arose in the context of the ongoing prosecution of [the original target] for drug offenses. References to drugs, therefore, could not be avoided.”).

Accordingly, it is proper for the Government to argue that the Defendant’s obstruction, concealment, falsification of records, and false statements were motivated by a desire by the Defendant to hinder and frustrate

the investigation into the Defendant's actions, even though we cannot fully know the nature and extent of the Defendant's actions. The Government may properly argue to the jury that we do not know the precise manner and circumstances of Tur's death, or whether the Defendant contributed to it, at least in part because of the Defendant's obstructive conduct. The Government may also properly argue that the resulting lack of answers regarding what happened to Tur was the specific intent of, and not an unintended byproduct of, the Defendant's obstruction, concealment, obstruction, concealment, falsification of records, and false statements.

The jury is certain to wonder what happened to Tur—why he was bleeding, how he ended up in the water, and why an otherwise able-bodied man drowned in water when most people could easily swim to shore. Preventing the Government from acknowledging that these questions exist at least in part due to the Defendant's actions ignores that the Government bears the burden of proving the charged crimes beyond a reasonable doubt, and runs the risk that the jury may be confused into thinking that the underlying question of how Tur died is relevant to its verdict, which it is not.¹⁶

¹⁶ Furthermore, if the Government is not permitted to make these arguments, the jury could be left with the perverse conclusion that the evidence establishes that the Defendant did murder Tur and that the Government is accusing him of that.

X. CONCLUSION

For the reasons stated herein, the United States respectfully submits that the Defendant's pretrial motions (Dkts. 31-32 and 34-36) should be denied.

Respectfully submitted,

/s/ Peter M. Nothstein
Counsel for the Government
TODD GEE
Deputy Chief, Public Integrity Section
Todd.Gee2@usdoj.gov
PETER M. NOTHSTEIN
Trial Attorney, Public Integrity Section
Peter.Nothstein@usdoj.gov
Criminal Division
U.S. Department of Justice
1400 New York Avenue, NW
12th Floor
Washington, DC, 20005
Telephone: (202) 514-1412

DATED: August 16, 2019

CERTIFICATE OF SERVICE

I HEREBY CERTIFY that on this date, I electronically filed the foregoing pleading with the Clerk of the Court using the CM/ECF system, which will send notification of such filing to the attorneys of record for the defendant.

Dated: August 16, 2019

/s/ Peter M. Nothstein
Todd Gee, Deputy Chief

Peter M. Nothstein, Trial Attorney
Public Integrity Section
Criminal Division
U.S. Department of Justice

1
2 TESTIMONY

3 OF

4 [REDACTED]
5
6 COMPLAINT NUMBER 2015R02127
7
8
9

10 [REDACTED]
11 THE BRYAN SIMPSON UNITED STATES COURTHOUSE

12 300 NORTH HOGAN STREET

13 JACKSONVILLE, FLORIDA 32202
14
15
16
17
18
19
20
21
22

23 TODD GEE, ESQUIRE

24 MARK J. CIPOLLETTI, ESQUIRE

25 US DEPARTMENT OF JUSTICE, PUBLIC INTEGRITY SECTION

1 Q. All right. Let's move to Monday, January the 12th.
2 When you came into work that Monday, January the 12th, did things
3 start to change with respect to the situation going on with
4 Guantanamo Bay?

5 A. Somewhat. You know, I arrived in early because of what
6 was going on, and when I arrived -- very soon after I arrived,
7 our inspector general [REDACTED] came to my office and said --
8 you know, he was -- I think he was made aware of what was going
9 on over the weekend, but he came into his office that morning and
10 there was -- he had received a report from somebody at Guantanamo
11 Bay, which had alleged that the commanding officer has -- was
12 allegedly having an affair with the wife of the person who was
13 recently found deceased and that there had been an altercation
14 that night at the officer's club -- the Friday night before at
15 the officer's club that -- you know, it alleged that Captain
16 Nettleton had been, you know, dancing closely with the wife and
17 there had been a verbal and kind of a screaming altercation
18 between them. So that changed the complexion a little bit.

19 Q. So you learned some of the -- sort of a summary of
20 some of the allegations in the IG report from your IG that Monday
21 morning?

22 A. Correct.

23 Q. Okay. And after you had that conversation with your IG
24 that Monday, did you also speak with Chris Nettleton that Monday?
25 I'm sorry. Captain Nettleton.

1 A. I'm sure I did throughout the course of the day, yes.

2 Q. And on that Monday, were those also sort of the same
3 types of logistical-type conversations you had with him the day
4 before after the body had been found?

5 A. Yes.

6 Q. About --

7 A. Yeah. I -- you know, when we are made aware of those
8 type of allegations, you know, an IG complaint, then what I had
9 done is I had talked to captain -- or, I'm sorry, [REDACTED]
10 who is the IG, about initiating an investigation on our own to,
11 you know, verify or, you know, disprove the veracity of the
12 claims. So he and I talked about him initiating an investigation
13 that would, you know, entail -- most likely entail him going down
14 to Guantanamo Bay for a few days to ask people some questions to
15 find out whether or not this was valid.

16 Q. Okay. And would that IG investigation be something
17 that Captain Nettleton would be anticipated to be a member of the
18 investigative team for that?

19 A. No, no, absolutely not, because he was -- you know, he
20 was allegedly involved in it. So it would have been -- he would
21 not have been included in on it. He would have obviously found
22 out about it once somebody came down and started asking
23 questions, but it wasn't something that I made him aware of in
24 any of our conversations throughout the week, because, you know,
25 again, I didn't want to taint the -- kind of the investigation,

1 or if he was doing the things that he was alleged to be doing and
2 then I told him we were coming to investigate, then he could
3 potentially interfere or in some way try and modify, you know.

4 Q. And maybe it's part of that; just for those of us that
5 aren't in the Navy or may not be very familiar with the IG
6 process, you know, that Monday you learn about this IG complaint
7 and you speak to Chris Nettleton that Monday about logistics and
8 things like that. Why -- in your mind, like, why would you not
9 be like, you know -- I'm sorry. Captain Nettleton. Why would
10 you not then ask him, like, have you had an affair and all these
11 sorts of things; why is it that you wouldn't do that after having
12 receiving an IG report?

13 A. Well, because he reported to me. Myself and the
14 admiral we're his next level chain of command. So if there were
15 a disciplinary issue for him, then we would be responsible for
16 administering military justice against Captain Nettleton.

17 So, for me, I had to be very careful in my discussions
18 with him, because if I were to have begun probing him and asking
19 him those types of questions without first reading him his
20 rights, then that information -- any information that I might
21 have asked him or that he might have revealed, I wouldn't have
22 been able to use then in a subsequent military -- you know, a
23 disciplinary proceeding. So I had to be very careful.

24 Q. So that's why after learning about the IG report that
25 Monday, you're not probing him asking him a whole bunch of

1 questions about the IG report?

2 A. Exactly.

3 Q. Let's move on to the next day, Tuesday the 13th. Did
4 you speak with Captain Nettleton on that Tuesday the 13th?

5 A. I did.

6 Q. Okay. And, as best you can remember, tell us what it
7 is that you believe occurred in those conversations with him on
8 that Tuesday.

9 A. Well, you know, I think it was around mid-morning I got
10 what I deemed to be a -- it was a bit of a -- it was a little
11 strange call. He seemed to be a little bit rattled, and he said,
12 hey, you know, I want to let you know there's some crazy rumors.
13 I was out and about doing my work in the community and a couple
14 of folks that I know stopped me and said there's these crazy
15 rumors about me having an affair with the wife of this individual
16 who showed up dead.

17 And, you know, it's a small community here. There's no
18 where to go. You're in a fence in a foreign country that you
19 can't go out into it. So it's very close-knit community.

20 And he said, you know, rumors spread quickly here, but,
21 you know, hey, I want to let you know there's absolutely no truth
22 to this rumor. I was not having an affair. None of this is
23 going on.

24 But he was very concerned because he -- because of the
25 position that I talked, his position as the commanding officer,

1 and being responsible for military justice and security and
2 safety and all of things, and, you know, kind of the military
3 very high standards about, you know, conduct of a commanding
4 officer, he felt very uneasy given the circumstances of the death
5 and now these rumors. He felt like it put him -- you know, shed
6 a bad light on him. And, frankly, it's not unusual for
7 commanding officers in those circumstances to be relieved of
8 their duties because of things like that.

9 Q. And, Captain, did he -- in relaying to you these rumors
10 that he had overheard circulating or had learned on the island
11 about him having an affair with Mr. Tur's wife, what did he say
12 with respect to the veracity of those rumors?

13 A. He said unequivocally that they were not true. There's
14 no truth to them, period.

15 Q. Okay. And in this conversation with you where he is
16 relaying sort of the rumors on the island to you -- in that
17 conversation on approximately that Tuesday in this rumor
18 conversation, we'll call it, did he relay to you any information
19 about either rumors -- or, actually, did he relay any sort of
20 information to you at all about Chris Tur having been at his
21 house or himself being in any kind of physical or verbal
22 altercation with Chris Tur before his death?

23 A. No, none whatsoever.

24 Q. So he doesn't even relay that information as a rumor on
25 the island at that point?

1 A. No.

2 Q. And, additionally, in these conversations that you were
3 having with him that Tuesday, was there also references to an
4 e-mail that contained other rumors that were about Mr. Tur's
5 death?

6 A. Yeah. At some point during the day, he talked about an
7 individual who had previously served in some capacity on the
8 island, and he relayed to me that this individual -- I believe it
9 was his -- or he had gotten in trouble or his family members had
10 gotten in trouble, and the end result of a series of disciplinary
11 events that happened Captain Nettleton in his role again as kind
12 of the CO of the base made the decision to force him to leave the
13 island and to leave his job because he was a disciplinary issue
14 for him.

15 That's -- it's not completely unusual. It doesn't
16 happen a lot because, you know, obviously it has big
17 ramifications for an individual. So, generally, if you're
18 kicking somebody off your base -- and in some cases that entails
19 them being fired from their job -- it's got to be something
20 that's pretty substantial for that very reason.

21 So he said that this guy had been a problem in the past
22 that he ended up investigating and took the action of kicking him
23 off the island because of his behavior. That since then, this
24 individual had on occasion, you know, sent e-mails to the chain
25 of command and others to try and, you know, seek some retribution

1 for him getting kicked off the island.

2 So he mentioned that this guy had sent him an e-mail
3 and was making, you know, kind of some veiled accusations, that
4 kind of thing. He eventually sent this to me, which, again, I
5 took to be part of, hey, these are the wild rumors that are going
6 around.

7 Q. Okay. And so Grand Jury Exhibit Number 2, is this the
8 series of e-mails that ultimately get forwarded to you and
9 Admiral [REDACTED] that you guys are back and forth communicating
10 about from this individual Mr. [REDACTED] that he had sent Captain
11 Nettleton that he also spoke with you about that Tuesday?

12 A. Yes.

13 (Grand Jury Exhibit Number 2 was marked for identification.)

14 BY MR. GEE:

15 Q. And in your conversations with Captain Nettleton, he
16 sort of, as you described -- he sort of described the background
17 on this person and how they relayed things before, you know.
18 Based on your conversation with him, what impression did he leave
19 you with about the veracity of this person?

20 A. He -- again, he said unequivocally there's no truth at
21 all to any of these rumors.

22 I can tell you that. I'm perfectly comfortable with
23 that, but at the same time he was a little bit rattled. He was a
24 little shaken because of the potential ramifications of this.
25 And so, again, in my role as the chief of staff and as a mentor,

1 I said, you know -- JR was his kind of call sign. I said, JR,
2 you can't really worry about this stuff. You've got a lot to
3 take care of. You've got to take care of this family. You've
4 got a lot of work to do to organize getting folks there.

5 Now we were talking about bringing additional NCIS
6 agents in from off the base and how are we going to get the body
7 and the family back to the states to the location that they're
8 eventually going to want to go to have a funeral, and assisting
9 them with all those other things. So, you know, I just tried to
10 reassure him that, you know, now there's an investigation going
11 on. You know, you're telling me it's not true. Great. That's
12 all going to come out in the wash in this investigation. I said,
13 you can't really focus on that right now. You need to focus on
14 doing your job as the commanding officer and make sure you're
15 doing everything you can to not only take care of the family,
16 but, you know, you have a wider community now. There's a 5,000
17 person community there that's very isolated, very insular, and
18 close that you need to insure that the community is taken care of
19 as well.

20 Q. And you talk about how he was rattled in these
21 conversations you were having with him on Tuesday where he was
22 relaying to you the rumors. You're, of course, speaking to him
23 on the telephone; correct?

24 A. Yeah.

25 Q. What was it that you could tell about him in the

1 conversation that -- what is it that was occurring on the other
2 end of the phone that made you feel like he was rattled, as you
3 say?

4 A. Well, he just sounded very nervous and I -- in the
5 course of the conversation it came out, you know -- I think he
6 conveyed something like, hey, you know, I've got a guy that's --
7 who showed up dead here and now there's these allegations that
8 I'm having an affair and so it puts me in a very bad position as
9 a commanding officer because -- and I know the history in the
10 Navy and commanding officers often get relieved for these kind of
11 things. So it scares me that these false rumors are out there,
12 because it has implications for my job as the commanding officer
13 and potentially my career.

14 Q. And, now, in this e-mail that he ultimately forwards to
15 you and Admiral [REDACTED] later that evening or that afternoon --
16 it looks like he forwards it to Admiral [REDACTED] first at 4:39
17 p.m. and then you get looped in a little bit later on that
18 evening on some of the e-mails by about that evening.

19 In the original e-mail that sort of -- the e-mail from
20 this person Mr. [REDACTED] to Captain Nettleton he says, I know you
21 will do your best to get to the bottom of the situation. I have
22 also heard someone previously got into a fight with a deceased
23 person at the Bayview over some alleged sexual harassment of the
24 victim's wife. Have you heard about his too?

25 In your conversations with Captain Nettleton that

1 Tuesday when he's talking about the wild rumors and so forth, did
2 he provide -- in the wild rumors conversations that Tuesday, did
3 he make any references to the Bayview or the fight with the
4 deceased person or alleged sexual harassment of the wife?

5 A. No, he didn't.

6 Q. So most of your conversation about this Mr. Stone
7 person was basically him describing the veracity and the
8 background on this person?

9 A. Yeah. Saying there's absolutely no truth to it, and,
10 essentially, to me he led me to believe that Stone was somebody
11 with an ax to grind who had gone after him before and he -- you
12 know, Captain Nettleton had, essentially, said, hey, he's done
13 this kind of before. He's made allegations against me because
14 he's, you know, seeking retribution, and I'm talking to NCIS and
15 the Jags here about what I can do to stop him from harassing me,
16 that kind of thing.

17 Q. Now, turning to the next couple days of that week, so
18 Wednesday, Thursday of that week following the finding of the
19 body. Do you believe that that Wednesday, Thursday, that
20 sometime in that timeframe you may have had another conversation
21 with Captain Nettleton where he started to provide you with more
22 information than in the past?

23 A. Yeah, he did.

24 Q. Okay. And you believe that that conversation was on
25 either Wednesday or Thursday?

1 A. I think it was late Wednesday, early Thursday. I can't
2 remember exactly.

3 Q. Okay.

4 A. It was towards the end of the week.

5 Q. And tell us how that conversation occurred and, as best
6 you can, what was said in that conversation.

7 A. Well, he called and, you know, got a note that, hey,
8 Captain Nettleton is calling for you, and so I presumed it was
9 just a -- kind of an update on what's going on.

10 He was on the line and he said, hey, I need to call you
11 and tell you something. He said that, you know, I wanted to
12 provide some additional information; that there was, essentially,
13 a going away party -- an event at the officer's club on Friday
14 night and during that -- that he was there and the Turs were
15 there and a number of other folks were there.

16 During that conversation -- or during that event, he,
17 essentially, said that he had gotten into a verbal altercation
18 with the deceased, Chris Tur, over some accusations from him
19 directly that he had been sleeping with his wife, having an
20 affair with his wife, and that his behavior during the -- during
21 his time at the O'Club was kind of cyclical. It would go from
22 one minute to, hey, you're screwing around with my wife kind of
23 poking you in the chest aggressively going after him to kind
24 of -- to back to the other end of the scale that said -- where he
25 was saying, hey, you're a great guy. I really like you. You're

1 a good CO.

2 You know, so it added a different -- a new and
3 different dimension to the situation. He said later, you know,
4 the party broke up at the -- it kind of was settled down, the
5 argument settled down, and everybody kind of went their own way.
6 Then later some time that evening that he eventually showed back
7 up to his house and kind of did the same thing. You know, I --
8 hey, you're screwing around with my wife. Why are you doing?
9 That, you know, kind of angry to back, you know, cyclical that I
10 really like you. You're a really great guy. You've done a great
11 job here as the CO.

12 So he told me these things, and he said they kind of
13 had some discussions at his house -- at the front door of his
14 house, which after some amount of time was kind of again tamped
15 back down -- his concerns were tamped back down. He denied them.
16 At some point, you know, he said Chris Tur wanted to come in, but
17 he said he wouldn't allow him to do that because his daughter was
18 in the house, and at some point Chris Tur left the house.

19 Q. And in this conversation, did he -- did he say to you
20 where Chris Tur went after he left his house or which direction
21 he was going or anything like that?

22 A. Well, there's only one direction because his house is
23 on the end of a peninsula. It's on the very tip of a peninsula,
24 so the only direction you can go is back down, unless he was
25 going to jump in the water.

1 Q. Well, leaving aside your sort of knowledge of the
2 geography, in that conversation with him, did he have a specific
3 recollection of where he --

4 A. No, he just said he left.

5 Q. He just said he left.

6 A. Yeah.

7 Q. And focusing on just a couple parts of that
8 conversation, Captain. First, as he's relaying these additional
9 details to you in this conversation, are these additional details
10 about the argument at the Bayview and the -- and Chris Tur coming
11 to his house, when you're hearing these details on that -- in
12 that Wednesday or Thursday call, is this -- in your mind, were
13 you recognizing these are not details he has provided me before?

14 A. Yeah. I was absolutely shocked, to be honest, because
15 it was 180 degrees out from what he had been providing. You
16 know, he had -- all along he had relayed this information as if
17 it was second or thirdhand reports that he was receiving from
18 others. And, now, you know, we're -- Saturday, Sunday, Monday,
19 Tuesday, Wednesday, Thursday -- five-ish days into this and he's
20 telling me not that he -- he's admitting to me that, although he
21 led me to believe that he was getting these reports, he had
22 direct first-hand knowledge of it. So, you know, my jaw dropped
23 to my belt line, you know.

24 Q. And in addition to him providing -- you know, in your
25 mind, after you got off the phone with him or during the phone

1 call, in addition to sort of there being a difference in his
2 description of where information is coming from -- so now some of
3 it is information of things he saw and witnessed himself, the
4 additional details about himself being in a fight and Mr. Tur
5 coming to his house, those were not details that had -- were
6 those details that he relayed to you in any fashion --

7 A. No.

8 Q. -- previously, whether it was I, myself, saw these
9 things or other people are saying these things happened; those
10 two details were new to you?

11 A. Yes.

12 Q. And in this conversation, where is it that he -- well,
13 in this conversation where he's describing to you how Mr. Tur
14 came to his house, did he -- was it -- did he make it clear to
15 you that the conversation occurred outside of his house?

16 A. That was my impression that -- from what he said was
17 that -- because I remember him saying something along the lines
18 of that he wanted to come in and talk about it, and he said, no,
19 I talked to him about it at the front door. And so, yeah, he
20 didn't come in the house. He led me to believe that he was not
21 in the house.

22 Q. And did you ask him -- in this conversation with him
23 where now he's providing these additional details, did you ask
24 him or did he say -- was there a relaying of why all this is just
25 coming out now; an explanation of why this is coming out now?

1 A. Well, again, I had to be very careful from the military
2 justice that I didn't start -- because, you know, as an
3 inquisitive person, I wanted to start going -- I wanted to get
4 some of the details, but I couldn't do that because I would have
5 been soliciting information that then would have been tainted for
6 any type of military justice.

7 So -- but, you know, to me, again, it was shocking
8 information. I, you know, said, why didn't you tell us this
9 before.

10 And he said, well, I didn't say it because I just
11 didn't think it was particularly relevant, which, again, caused
12 my jaw to drop because I thought, well, wait a second, you've got
13 a guy who's missing and dead and you potentially were now the
14 last person to see this guy, you know. I'm thinking to myself,
15 how do you think that this wasn't relevant to what's going on.

16 Q. And so his explanation was that he didn't think it was
17 relevant; that was the word he used?

18 A. Exactly.

19 Q. And, to be clear, Captain, in this conversation when
20 he's providing you the explanation of why he's providing these
21 different details, did he say anything about not providing the
22 details because he was embarrassed or concerned about his career?
23 Was that any part of the explanation?

24 A. No. He just said, I didn't mention it because I didn't
25 think it warranted being mention.

1 Q. And in this conversation, even when he's providing you
2 these additional details you've described, did he ever mention
3 there being a physical altercation with Chris Tur in that
4 conversation, a physical altercation?

5 A. Well, I think at the -- yes, at the O'Club. There was
6 that altercation at the O'Club, but he never indicated in our
7 conversation that there was a physical altercation at the front
8 door or anything along those lines.

9 Q. Okay. And, as best you can remember, what was it
10 that's described that was physical at the O'Club?

11 A. Well, you know, what I recall was that, you know, he
12 confronted him -- confronted him, got in his face kind of -- I
13 don't know whether -- I don't think there was any blows thrown,
14 but there may have been some close interaction and pushing and
15 shoving. That's my impression, you know.

16 Q. Okay. And -- but he never said anything physical
17 occurred at his home?

18 A. No.

19 Q. Okay. Fast-forwarding, Captain. Is it accurate to say
20 that in the weeks and months that followed you as sort of the --
21 you eventually became the commander of Guantanamo Bay on a
22 temporary basis; is that right?

23 A. Correct.

24 Q. And, also, as chief of staff to Admiral [REDACTED] as
25 well; correct?

1 A. Correct.

2 Q. And so in performing those roles, was there certain
3 information that you learned about what NCIS was uncovering
4 during the investigation?

5 A. Yeah. I was intimately involved in it because,
6 again -- because I was the chief of staff and I was more senior
7 and more experienced and had had command of an instillation and
8 had the experience and the knowledge, and then going into a
9 situation that was unfolding at Guantanamo, Admiral [REDACTED]
10 wanted to have somebody who was kind of seasoned and knew what
11 they were doing. So she sent me to take over. Eventually, when
12 the decision was made to relieve Captain Nettleton, she sent me
13 down there because I, you know, kind of knew the ropes.

14 Q. And so, for example, in those roles, did you learn that
15 the investigators had found Chris Tur's blood inside Captain
16 Nettleton's home?

17 A. I did. They -- NCIS asked for -- as the investigation
18 began to unfold and they started interviewing folks and it led
19 them in various directions, you know, I provided search warrants
20 for the property and various locations to facilitate the
21 investigation.

22 In the course of that investigation, we learned that
23 there had been, in fact, an altercation in the house, that a very
24 large amount of blood was discovered to have been in the house
25 that had been clearly attempted to be cleaned up, that there were

1 missing items from the house -- so, yeah, pretty...

2 Q. And did you learn that Captain Nettleton's daughter
3 reported actually seeing Mr. Tur inside the home?

4 A. I did. Part of the -- one of the things that they did
5 was they searched all the cell phone records, and, at some point
6 during that evening, her -- Captain Nettleton's daughter had text
7 a friend and --

8 Q. That's okay. I don't need you to report on it, though,
9 but part of the text information that was relayed to you that
10 Mr. Tur had been inside the house?

11 A. That's right. That there was an altercation going on
12 inside the house. That there was a lot of noise downstairs. She
13 peeked around the corner and saw --

14 Q. Saw Mr. Tur and other details?

15 A. Right. Exactly.

16 Q. And when you learned this information from the
17 investigators that there had been -- that there was blood in the
18 house, that it had been cleaned up, that his daughter had
19 reported seeing Mr. Tur inside the house, when you learned those
20 facts, what in your mind, if anything, did you think about those
21 facts in comparison to what he told you in this
22 Wednesday-Thursday conversation when he was describing Mr. Tur
23 coming to his home; was there anything that, in your mind, when
24 you learned those facts stuck out that is different than what he
25 told me before?

1 A. Well, it was clearly different. By that time, I --
2 yes, it stuck out to me as here's another example of an attempt
3 by him to not fully tell the truth or not -- or to kind of tell
4 the different version of what happened, because he specifically
5 told me, hey, I turned him -- we had a discussion at the front
6 door and I turned him away and told him, you know, to go home.
7 So, now, you know, the next day or, you know, over the next
8 couple of weeks I found out that that as well was untrue.

9 Q. Okay. Now, after this conversation with -- we're going
10 to have to move through things a little bit quickly, but after
11 this conversation with him that Wednesday or Thursday when he's
12 providing you a lot of these additional details for the first
13 time, did you have another conversation with him after that later
14 that week -- so, you know, Thursday, Friday, something like
15 that -- where he conveyed other odd information or discussed odd
16 proposals to you, something that stuck out in your mind?

17 A. Yes. You know, late in the week -- you know, again, it
18 might have been later Thursday or Friday -- he was clearly very
19 concerned at that point and he -- you know, he -- it was kind of
20 a strange call as well.

21 He said to me, hey, I know this sheds a bad light on me
22 as the CO. It sheds a bad light on the Navy because of these
23 untrue rumors that are going around. He said, I know how it
24 looks. It's absolutely not the case. None of it is true. But,
25 you know, maybe I should resign; maybe you guys should just let

1 me resign as the commanding officer and, you know, kind of walk
2 away from this thing and that way it doesn't shed a bad light on
3 the command and on the Navy.

4 You know, at that point, I essentially said to him,
5 look, it is what it is at this point. There's an investigation
6 underway. That investigation has to play out now, and so for you
7 to resign really would be moot. We have to just let it play out
8 and see where it goes.

9 So at end of the conversation it was, you know, let's
10 just kind of wait and see. We're in a wait and see mode and let
11 the investigation go.

12 Q. Okay. And, now, fast-forwarding to the following week.
13 So this would be the week of that included January 21st, so a
14 full week after the body has been found.

15 By early -- sometime early that week, did Admiral
16 [REDACTED] notify Captain Nettleton that he was going to be removed
17 from command in Guantanamo?

18 A. Yes. You know, I was getting reports over the weekend
19 from NCIS, you know, updates of their investigation. Obviously,
20 the information that he relayed to me that kind of corroborated
21 the IG, you know, all the other stuff that had gone on. Yeah,
22 that got to the point where Admiral [REDACTED] as his boss, was
23 taking very seriously the fact that, you know, hey, I got a
24 commanding officer of an overseas isolated instillation who is
25 alleged to have had an affair, which that in and of itself might

1 warrant his relief as the commanding officer, but now there's a
2 missing person and a person who's shown up dead that he is
3 implicated -- potentially implicated in. You know, the
4 inevitability of it was building throughout the weekend.

5 When -- you know, when I left on Friday, we kind of had
6 agreed that we would get updates through the weekend and then
7 early the following week see where we were and make a decision.
8 So came into work on Monday, had another brief of where we were,
9 and Admiral [REDACTED] was at the point where she felt like she
10 needed to relieve Captain Nettleton because of all the things
11 that were going on. So it's not as simple as her picking up the
12 phone and saying, hey, you're relieved.

13 Q. Sure.

14 A. We've got to prep our chain of command, which goes up
15 to Washington, D.C., and they have to have time to notify
16 congress. Then it comes back down, okay, we've done it, and now
17 you can do it. So that -- you know, I believe it was Tuesday
18 mid-day she ended up calling him and telling him that she was
19 going to relieve him.

20 Q. And then after he was relieved, did you have another
21 conversation with him about his -- did you, yourself, have a
22 separate conversation with him after he was relieved in which he
23 references his resignation or anything like that?

24 A. I did. He -- as that was -- you know, he essentially
25 said to me afterwards he said, hey, you know, I offered to resign

1 last week. Is there any way we can kind of work that? I mean,
2 can I not be relieved? Can you just let me resign?

3 And I said, no, I can't. That's -- you know, that's
4 not possible at this point. You know, you've got to be --
5 Admiral Jackson has lost confidence in you and it is what it is
6 and...

7 Q. Okay. And just jumping forward for a little bit, by
8 approximately January 21st -- on or about January 21st, you were
9 sent to Guantanamo Bay and you flew there; correct?

10 A. Correct.

11 Q. And when you got there, did you encounter Captain
12 Nettleton?

13 A. I did just very briefly. I got off the airplane, and
14 he was waiting to get on the airplane in the air terminal. So I
15 went in and I met with him, you know, for 10, 15 minutes max. It
16 was, you know, by then all a done deal. So the tone of the
17 conversation was, hey, keep your head up. Again, if what you're
18 saying is true, none of this is true, then it's all going to come
19 out and you'll be exonerated. So it wasn't -- I wasn't going to
20 try to further beat him down, but I was just trying to tell him
21 to keep his head up.

22 You know, obviously, these are situations where you
23 don't know how folks are going to react. So one of the things
24 that I had done prior to him actually getting the call that he
25 was going to get relieved was to call the executive officer

1 Commander [REDACTED] and tell him it was going to happen so that he
2 knew so that if, you know, Captain Nettleton tried to do
3 something crazy that I had somebody there watching him, you know.
4 You never know. I mean, he could have gone to the armory. You
5 don't know what's going to happen.

6 Q. And so in that conversation with him at the airport,
7 did he provide you any additional details about his interactions
8 with Mr. Tur or anything like that?

9 A. Not that I recall; just more of the same.

10 Q. And in any of your conversations with him from the day
11 that he first reported that Mr. Tur was missing all the way until
12 the airport conversation until you saw him at Guantanamo Bay and
13 he got on the plane, did he ever in any of those conversations
14 tell you there had been a physical altercation at his house with
15 Mr. Tur?

16 A. No.

17 Q. Would that have stuck out in your mind had he told you
18 that?

19 A. Absolutely.

20 Q. And, Captain [REDACTED] just briefly, can you explain to the
21 grand jury what a commander of Guantanamo Bay like Captain
22 Nettleton what a command investigation would be; give us some
23 examples of what a command investigation would be versus an NCIS
24 investigation?

25 A. Yeah. We generally will do command investigations

STATEMENT

Place : Building 919, Naval Air Station Jacksonville

Date : June 5, 2015

I, RDML [REDACTED], USN, make the following free and voluntary statement to Special Agent [REDACTED] whom I know to be a Representative of the United States Naval Criminal Investigative Service. I make this statement of my own free will and without any threats made to me or promises extended. I fully understand that this statement is given concerning my knowledge of the death of Christopher TUR, Civ onboard Naval Station Guantanamo Bay Cuba.

For identification purposes, I currently hold the rank of Rear Admiral in the United States Navy and am assigned as Commander, Navy Region Southeast, located onboard Naval Air Station, Jacksonville, FL. In this capacity I am directly responsible for numerous U.S. Navy Activities in the Southeast Region, both CONUS and including Naval Station Guantanamo Bay, Cuba. I graduated from the United States Naval Academy in 1988.

[Handwritten signature]

NOT USED

At approximately 2200, Saturday 10Jan15, I first learned that Christopher TUR, a civilian employee of the Navy Exchange onboard Naval Station Guantanamo Bay, Cuba was missing, after reading an email from my blackberry I had received from CAPT J.R. NETTLETON, USN, who was the Commanding Officer of Naval Station Guantanamo Bay, Cuba. NETTLETON reported directly to me.

NETTLETON sent the email notification to me around 2000 but I did not see/read it until 2200.

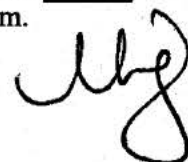
Because my cell phone is not international, I was not able to return calls to GTMO. This meant that if I wanted to talk to someone at GTMO, I needed to email them to call me, or call my ROC and ask them to call the base to direct the CO to call me. Subsequently, NETTLETON and I emailed each other back and forth a couple of times that evening where I asked him for additional details surrounding Mr.

TUR's disappearance. NETTLETON provided me with some information related to the background and history of the TUR family in his response. Essentially, NETTLETON indicated that TUR has gotten drunk before and disappeared for a night. (At this time, NETTLETON did not share with me the details that I learned from him and NCIS several days later following the recovery of Mr. TUR's body. Specifically, I later learned on Monday and Tuesday (2 days after the incident) that Mr. TUR went missing following a command hail and farewell function at the Club and an altercation at the NETTLETON's home during which Mr. TUR accused NETTLETON of having an affair with TUR's wife, [REDACTED]. I have provided copies of the email correspondence that I had with NETTLETON on the night of 10Jan15 to Special Agent [REDACTED] which completely detail the content of our

correspondence. On 10 Jan 15 I ended my email correspondence with NETTLETON by instructing him to phone me the next morning, Sunday 11Jan15, which he did at approximately 0800. During that phonecall NETTLETON provided me with details pertaining to land and sea searches. NETTLETON indicated that TUR's wife was worried but ok, and that they were going to continue the search. After the morning phone call with NETTLETON, I travelled to Washington D.C. for business. When I landed, I saw that I had a missed call from NETTLETON. I emailed him to call me back, and at approx 1630 EST NETTLETON called me back to tell me via phone that the deceased body of TUR had been recovered in the water by the Coast Guard. He stated during this phone call that the body had indications of possible "marine bites", but no other apparent injuries. I asked NETTLETON if his wife, [REDACTED] was there to help Mrs Tur, and NETTLETON shared with me that his wife was off island taking care of a sick parent. NETTLETON and I talked about the next necessary steps with respect to the medical examiner and death notifications. Within the hour of talking to NETTLETON, I called my boss, CNIC, and left a phone message that the body had been found. At this point, I had no indication that there was any command involvement leading to this death, and I was unaware that there had been a command function and an altercation between TUR and NETTLETON. I was aware that NCIS would initiate an investigation into the death as required for all deaths on Naval Installations.

On Monday, 12Jan15, I attended a Flag Officer meeting at the Pentagon in which cell phones were not allowed. I checked my phone at lunch and had a message to call my Navy Region Southeast Inspector General, Mr [REDACTED]. I called Mr [REDACTED] and he informed me that there was an anonymous IG Hotline complaint that had come in that accused NETTLETON of having a romantic relationship with [REDACTED] TUR. In that phone conversation with my IG, we discussed the fact that NCIS was already involved and would be sending a group of agents to GTMO as soon as possible to investigate the death. We discussed whether or not I should direct a command investigation to run concurrently with the NCIS investigation to look specifically into the allegation of a relationship. After consulting with the IG, I decided that the NCIS investigation would address all of the allegations. I did not speak with NETTLETON on 12Jan15. Later that day after the meetings, after reading an End of Day email from my Chief of Staff, I spoke with the Chief of Staff, CAPT [REDACTED] USN who informed me that he had spoken to NETTLETON several times on Monday. [REDACTED] stated that NETTLETON was surprised by the accusation of an affair with Mrs. TUR. NETTLETON also informed CoS [REDACTED] that Mr. TUR went missing after a command event in which NETTLETON was in attendance. This was the first indication that TUR went missing at or after a Command Event. In the End of Day email, CoS [REDACTED] wrote that he had directed NETTLETON to go find NCIS and be completely open with them.

page 2 of 5



On Tuesday 13Jan15, I directed NETTLETON to call me. During this phonecall NETTLETON told me for the first time that there was a command event on Friday 09Jan15 that he attended at the club and that it was a Hail and Farewell for his outgoing Executive Officer. NETTLETON acknowledged that there was a lot of drinking at this event, and there had been an argument between Christopher and [REDACTED] TUR inside the event and also in the parking lot. NETTLETON informed me that once the argument got settled down, he wanted to have people come back to his house after the event, but the new XO advised against it. NETTLETON told me that after he witnessed the argument in the parking lot between Mr. TUR and [REDACTED] TUR and after XO told him he should go home, he returned home alone and was home for approximately 30 minutes before Mr. TUR arrived at his house unannounced. NETTLETON indicated that he and Mr. TUR had a verbal argument that was overheard by his teenage daughter, [REDACTED]. NETTLETON told me that [REDACTED] heard Mr. TUR yell "you're fucking with my wife". NETTLETON classified Mr. TUR's behavior at his house as a "roller coaster" where one minute Mr. TUR was irate and then another acted like his best friend. NETTLETON reported to me that Mr. TUR left the COs home and went back to the club. NETTLETON assured me that the allegations Mr. TUR made toward him were untrue and that he did not have a relationship with [REDACTED] TUR. After hearing these details from NETTLETON, I told him to go find NCIS and explain everything to them as you just did to me. While I did not directly order NETTLETON to report this information to NCIS, it was delivered in the context that I expected he would have gone to NCIS.

On Wednesday, I continued to receive updates from my staff back in Jacksonville. I had been providing verbal updates to my boss, VADM [REDACTED] and on Wednesday, provided a written summary email, which I have provided to NCIS. This email reveals all of the above, and also specifically stated that at this point I had not yet lost confidence in CAPT NETTLETON to command. That said, I was getting more and more uneasy about the information that was being revealed. I returned to Jacksonville on Thursday, 15 January and was provided updates from my SJA and IG. They informed me that NCIS was at Guantanamo Bay and taking statements from as many people as possible and would continue to work through the weekend. I worked with my COS to make sure the arrangements for family members to travel to the island were in place, coordinated with NEX as Mr TUR was an NEX Employee, and also ensured that the planning for the memorial service was tracking. I did not speak with NETTLETON on Thursday as I knew that ASN [REDACTED] was visiting GTMO, and NETTLETON was focused on touring the Secretary around the installation.

I spoke on the telephone with NETTLETON on Friday 16Jan15 after Secretary [REDACTED] had left, during which NETTLETON asked me if he should step down as the Commanding Officer due to the allegations that were circulating that led him to believe the Guantanamo Bay community had likely lost confidence in him. My JAG, CDR [REDACTED] sat in on that phonecall meeting. At the time, I informed NETTLETON that I had not lost confidence in him. I told him that I was waiting for further information from NCIS regarding the investigation. I knew that investigative activity was scheduled to occur over the weekend which would provide some additional perspective and make the situation more clear. NETTLETON asked my advice on whether he should seek legal counsel, and I told him and my JAG, CDR [REDACTED], told him that we could not advise him on whether he should talk to an attorney but that he still had his JAG available to consult with. NETTLETON sounded very tired during this phone call, and I instructed him to get some rest and to focus on the mission and to ensure his command responsibilities were met with respect to his Installation CO mission and Mr. TUR's memorial services scheduled for the following day. I asked the CO and/or XO to provide me an update regarding the outcome of the services.

page 3 of 5



I received an email from the Executive Officer on Saturday 17Jan15 following Mr. TUR's memorial service as I had requested, and he advised that the Service went well. NETTLETON also sent an email that told me that he spoke with his father, who I know to be an attorney, and that he got a good night sleep and was feeling much better. Later in the day, I was informed by my SJA and CoS that NCIS had learned that there was a possible physical altercation at NETTLETON's home on 09Jan15 between NETTLETON and Mr. TUR and that there may be information about another potential affair between NETTLETON and someone previously assigned to a tenant command on Guantanamo. This was the first time I was told that the altercation at the residence was a physical altercation.

It became increasingly clear to me that there had been substantial elements of the event that occurred on Friday, 9 January, that had not been disclosed to me by the CO. Additionally, it also became clear to me that the CO had with-held information, had been participatory in a command event that included excessive drinking, had been in a physical fight with one of his staffmember's spouses, and that in some way he had contributed to and condoned a very unsafe situation that led to the death of a person at GTMO.

With new information and details coming in daily concerning NETTLETON and Mr. TUR throughout the weekend and Monday 19Jan15, I made the decision on Tuesday 20Jan15, to relieve NETTLETON as the Commanding Officer of Naval Station Guantanamo Bay, Cuba. I had to inform my chain of command and ensure all procedures were in place with leadership and media notifications, prior to actually relieving him.

On Wednesday, 21Jan15, at 1000, Skipper NETTLETON was directed to call my office. My JAG sat in on the call. I explained to him why I had lost confidence in his ability to command. I explained that he had withheld information, that he had behaved in a manner that was completely inappropriate for a Commanding Officer, especially at GTMO where he has to be in command all the time and maintain a safe situation. He acknowledged my remarks and indicated he understood. I acknowledged that he had asked on Friday if he could step down, but now based on the information I had, he was being relieved by me for loss of confidence. I indicated that at this point, knowing what I knew, that it would not be appropriate for me to allow him to step down. I explained to him that he was to return to Jacksonville that afternoon. My COS was already enroute and would be there shortly to assume duties as Acting Commanding Officer. I also told NETTLETON that I had been very impressed with the great work he had done in GTMO, that we would continue to support him and his family during this transition as we worked through the investigation, and that I was concerned about him.

Later that week on either Thur or Fri, I met with NETTLETON in my office upon his return from Guantanamo Bay, Cuba. NETTLETON stated that he understood why I needed to relieve him as the Commanding Officer and acknowledged that his behavior was not what I expected. NETTLETON told me that everyone will realize what happened and the truth will come out. Since NETTLETON's return I have had limited contact with him. During one meeting in which I provided him direction that I wanted him to work on Training instructions, we did discuss the status of his family, desires on where he could work (Pensacola vs Jacksonville) and also the status of his residence in GTMO still being under NCIS control. NETTLETON remains assigned to my staff. He is working out of the Chaplain's Office on base and has done some limited work on the Training tasker.

page 4 of 5



In response to a direct question asked of me during my interview, I hosted an "Icebreaker" reception on 05Nov15 at the T-Bar inside the Navy Gateway Inn and Suites onboard Naval Air Station Jacksonville, FL for ASN [REDACTED] since he was in town for the FFC Energy Day the following day. Commanding Officers were invited. Since the GTMO Industry day had been held on 5NOV and NETTLETON was in town, I invited him to attend the reception. NETTLETON was present at this evening reception. I knew everyone in the room, except the women working the bar. It is a small and confined space. While I have never met [REDACTED] TUR, I did not see her at this event. I left the event at approximately 1930 or 2000.

For documentation purposes, I considered NETTLETON as one of my higher rated installation Commanders. NETTLETON historically fully informed me relative to any matters requiring my attention. In the case of Mr. TUR's death, NETTLETON's response with respect to providing me substantive information was inconsistent with his prior habits. As I look back on it, he was willfully withholding information from me. He has yet to fully inform me of the extent of his interaction with Mr. TUR and his relationship with [REDACTED] TUR.

The aforementioned statement was prepared with the assistance of Special Agent [REDACTED] following my formal interview on 14Apr15. I have since read this statement and made any additional changes I deemed appropriate. I swear or affirm that this statement is a true and accurate account of my involvement in the events following Mr. TUR's reported disappearance and death, and is being provided voluntarily without any threats or promises having been made to me.

Sworn to and subscribed before me this 5 day of June in the year 2015 at

1015

Witnessed: 

[REDACTED]
Representative, Naval Criminal Investigative Service
AUTH: DERIVED FROM ARTICLE 136,
USMJ (10 U.S.C. 936) AND 5 U.S.C. 303

page 5 of 5

BLUESTAR FORENSIC

Latent bloodstain reagent

BLUESTAR® FORENSIC MAGNUM

A - Technical Application Note

The BLUESTAR® FORENSIC MAGNUM is a patented powerful new latent bloodstain reagent that has been designed to reveal blood, either fresh, dried or still humid, washed away blood, pure or diluted, in large or minute quantity.

The use of chemicals to investigate crime scenes is well known, and latent blood reagents such as Amido Black, Benzidine, Leucomalachite, Leuco Crystal Violet, Fluorescein, Luminol, Phenolphthalein, Herma-Glow, etc. have been applied for many years by forensic experts around the world to detect blood in minute quantity; blood cells still present in washed-away bloodstains; or blood otherwise invisible to the human eye.

Two main categories of such chemicals exist for this purpose:

- Those which react to the proteins and amino acids contained in the biological fluid: DFO, Amido Black, etc.
- Those which detect an enzymatic activity: Benzidine, Leucomalachite green, Leucocrystal violet, Luminol, Fluorescein, etc.

However, all these existing chemicals suffer from some major drawbacks.

Some are simply too toxic or too highly flammable to be used safely. Others compromise gravely or prevent DNA typing, are not sensitive enough, and are difficult to use. Still others are highly unstable, have a very short life, are difficult to maintain in storage, or any combination of the above...

To solve these problems, Professor Loïc Blum, Ph.D., a recognized authority in chemiluminescence, and the research team of the Enzymatic and Biomolecular Engineering Laboratory of the French National Center for Scientific Research (CNRS) at the University Claude Bernard of Lyon UCBL, France, developed the new latent bloodstain reagent BLUESTAR® FORENSIC MAGNUM.

When placed in contact with the iron contained in the heme nucleus of blood hemoglobin, the BLUESTAR® FORENSIC MAGNUM catalyzes (enzymatic peroxidase activity) and emits an intense blue (430 nanometer) chemiluminescence visible in the dark.

The BLUESTAR® FORENSIC MAGNUM can also produce a chemiluminescence when reacting to some household detergents such as bleach, or copper, but differences in intensity, emission spectrum, and reaction time allow for visual differentiation by experienced users.

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent is based on a new proprietary patented formula that is totally DNA typing compatible, extremely sensitive, stable, easy to use, and produces a very bright and long lasting chemiluminescence.

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent is extremely sensitive. Blood is detected on any background: material, animal or vegetable, in liquid or solid form. Blood may be detected either pure or diluted, while fresh, or long after it has been deposited (dried or even deteriorated). Furthermore, it allows for viewing the blood traces even if they have been rinsed away, or cleaned away (even on minute blood amounts).

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent produces such an exceptionally bright chemiluminescence that complete darkness is not required

for use, and no special photographic equipment necessary to record the evidence. Standard 35 cameras can be used with perfect results.

The BLUESTAR® FORENSIC MAGNUM latent bloods reagent is prepared within minutes. It is then applied lightly, in a mist, with a hand-held fine-spray bottle.

The blue chemiluminescence produced by BLUESTAR® FORENSIC MAGNUM latent bloods reagent is still visible several minutes after the bloodstains have been treated.

To facilitate crime scene investigation, BLUESTAR® FORENSIC MAGNUM can be applied numerous times over the same bloodstains without compromising the DNA typing.

B - User's Manual

1 - MIXING INSTRUCTIONS

Working solution ingredients

- 125 ml (4 fl. oz) of BLUESTAR® FORENSIC MAGNUM chemiluminescent solution
- 3 tablets

Active life

Best results are obtained when the product is used with 24 hours of mixing the oxidizer with the BLUESTAR® FORENSIC MAGNUM chemiluminescent solution. There is therefore no requirement to rush the investigation due to immediate product deterioration.

Mixing procedure

Open the bottle of BLUESTAR® FORENSIC MAGNUM chemiluminescent solution and pour its content into spray bottle (not included).

Add the 3 tablets.

Mount the plunger onto the spray bottle head and screw the head on the bottle firmly.

Allow about 1 or 2 minutes for complete dissolution and mixing of the chemicals, stirring gently with a circular motion of your hand. Do NOT shake the container upside down.

2 - APPLICATION INSTRUCTIONS

Lighting conditions

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent produces a very bright and long lasting blue chemiluminescence that does not require total obscurity to be visible. However, at high blood dilutions, the investigation will be much easier if the product is applied in total darkness. Furthermore, total darkness may allow the detection of tiny blood traces.

Indoors: Close all the windows, block all outside light sources, and turn off all the lights.

Outdoors: Wait for night time, and turn off all area lights in an urban environment. If necessary, screen off distant light sources, or even a very bright moon, and work facing away from parasite lights.

Wait for at least 5 minutes to allow your eyes to adjust to the darkness. Your pupils will dilate themselves and you will be able to better perceive the BLUESTAR® FORENSIC MAGNUM reaction.

Vaporization

In order to prevent biological contaminations of the revealed traces, wear personal protective equipment: safety goggles, gloves, dust respirator, protective clothing. Do not spray toward another person.

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent is designed to be vaporized from waist height in a

fine mist. Adjust the spray nozzle to obtain the finest mist possible. Very little of it is actually needed. Over-spraying does NOT result in improved blood detection, and in case the DNA is only available in very low quantities, over-spraying might dilute it too much for collecting exploitable samples, thus compromising its analysis.

Check how the product reacts by spraying BLUESTAR® FORENSIC MAGNUM on a testing sample. This test will also help you become familiar with the reaction on blood.

Spray lightly, horizontally ahead of you, at least 50 cm (2') away from the target, in a side to side sweeping motion, NOT pointing toward the ground.

Indoors: Be attentive not to saturate walls and vertical surfaces in order not to create drippings (as if you were spray painting).

Outdoors: Pay attention to wind direction, if any. Do not spray into the wind, but use it to carry a light cloud of product over the area to investigate.

Identifying "false" reactions

When reacting to blood, the BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent emits an intense light-blue chemiluminescence in the 420 to 440 nanometer range. However, "false" reactions may occur due to the presence of certain household detergents, chlorine, some paints and varnishes, copper, certain iron metabolizing plants such as lichens, thyme and some tree mosses, and certain soils containing iron.

Such "false" reactions are easily identifiable by the trained technician because their color, brightness, and duration differ from those of the typical reaction on human blood. Typically, "false" reactions are markedly dimmer and whiter.

"False" reactions due to chlorinated detergents are often interesting, because they may reveal attempts to wash or clean bloodstains, and to conceal a homicide.

3 - PHOTOGRAPHY

Photography of latent blood prints developed with chemiluminescence is not fundamentally different from regular daylight photography. The same four basic elements of photography (subject lighting, film sensitivity, aperture opening, and shutter speed) interact in exactly the same way.

Photography of BLUESTAR® FORENSIC MAGNUM detected blood prints is easy and produces excellent results.

Equipment

The chemiluminescence produced by the reaction of the BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent to blood is bright enough that no special equipment is needed. However, relatively long exposures may be needed for maximum picture quality and a tripod and flexible cable release are highly recommended in order to maintain the camera motionless during the time of the exposure. A 24 mm lens is recommended.

Film

No special film is required, but it is advisable to use low-light fast films in order to obtain acceptably short exposure time. A film speed of ASA 400 is suitable.

Lighting conditions

Total darkness is not required. Natural low intensity diffused light is preferred. Artificial light (tungsten or fluorescent) produces yellowish or greenish pictures. A flash should be avoided.

Instructions to obtain good pictures

Set the camera on a tripod, perpendicular to the area to be photographed.

Disable the automatic flash and the autofocus mode if the camera has one.

Use a large lens aperture, typically a f/2.8 "f/stop" value.

Set the exposure time to "B".

Focus the lens manually over a spot of light provided by a flashlight over the blood area.

Turn off the lights, but total darkness is unnecessary. Pictures shot in dimmed light will allow you to view not only the blood trace, but other details of the scene as well.

Re-spray the blood print to reactivate a bright chemiluminescence reaction.

Shoot several pictures using different shutter speeds, typically 30 seconds.

4 - COLLECTING BIOLOGICAL PRINTS

Samples of the revealed biological prints for subsequent DNA analysis are collected using the same methods as for any kind of biological prints.

5 - STORAGE, CLEANING & DISPOSAL

Storage

The BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent has a 3 year shelf life AFTER MANUFACTURING (expiration date imprinted on back of package) prior to mixing. If you wish to use the product after this date, we recommend that you perform a test to check the product performances.

Note: The product is warranted for 2 years after the DATE OF PURCHASE.

DO NOT attempt to store the product AFTER MIXING it with the oxidizing tablets. The mixed product is an active chemical compound that oxidizes. Inert gases are constantly released and will, in time, accumulate under pressure in a sealed container, causing swelling and leaking.

Cleaning

Since the BLUESTAR® FORENSIC MAGNUM latent bloodstain reagent is designed to be used on presumed blood on crime scenes, all precautions and regulations for the cleaning of the biohazards of blood apply.

Disposal

Dispose of unused BLUESTAR® FORENSIC MAGNUM solution in a sink under running water.

Dispose of cleaning residues in accordance with local, state, and federal regulations which apply to the biohazards of blood.

6 - MATERIAL SAFETY DATA SHEETS

Material safety data sheets (MSDS) for the BLUESTAR® FORENSIC MAGNUM bottle, tablets, and working solution are available in PDF format on our web site: www.bluestar-forensic.com/gb/download.php

BLUESTAR®
P.O. box 246
16 avenue de la Costa
Monte Carlo
98000 Monaco
+377 97 97 31 77
www.bluestar-forensic.com

Update 9.6 - April 2008
Ref. #BL-FOR-MAG

BLUESTAR FORENSIC

Latent bloodstain reagent

BLUESTAR® FORENSIC TABLETS

Ref. BL-FOR-TAB8

A - Technical Application Note

The BLUESTAR® FORENSIC is a patented powerful new latent bloodstain reagent that has been designed to reveal blood, either fresh, dried or still humid, washed away blood, pure or diluted, in large or minute quantity.

The use of chemicals to investigate crime scenes is well known, and latent blood reagents such as Amido Black, Benzidine, Leucomalachite, Leuco Crystal Violet, Fluorescein, Luminol, Phenolphthalein, Herma-Glow, etc. have been applied for many years by forensic experts around the world to detect blood in minute quantity; blood cells still present in washed-away bloodstains; or blood otherwise invisible to the human eye.

Two main categories of such chemicals exist for this purpose:

- Those which react to the proteins and amino acids contained in the biological fluid: DFO, Amido Black, etc.
- Those which detect an enzymatic activity: Benzidine, Leucomalachite green, Leucocrystal violet, Luminol, Fluorescein, etc.

However, all these existing chemicals suffer from some major drawbacks.

Some are simply too toxic or too highly flammable to be used safely.

Others severely compromise or prevent DNA typing, are not sensitive enough, and are difficult to use. Still others are highly unstable, have a very short life, are difficult to maintain in storage, or any combination of the above...

To solve these problems, Professor Loïc Blum, Ph.D., a recognized authority in chemiluminescence, and the research team of the Enzymatic and Biomolecular Engineering Laboratory of the French National Center for Scientific Research (CNRS) at the University Claude Bernard of Lyon UCBL, France, developed the new latent bloodstain reagent BLUESTAR® FORENSIC.

When placed in contact with the iron contained in the heme nucleus of blood hemoglobin, the BLUESTAR® FORENSIC catalyzes (enzymatic peroxydase activity) and emits an intense blue (430 nanometer) chemiluminescence, visible in the dark.

The BLUESTAR® FORENSIC can also produce a chemiluminescence when reacting to some household detergents such as bleach, or copper, but differences in intensity, emission spectrum, and reaction time, allow for visual differentiation by experienced users.

The BLUESTAR® FORENSIC latent bloodstain reagent is based on a new proprietary patented formula that is totally DNA typing compatible, stable, easy to use, and produces a very bright and long lasting chemiluminescence.

The BLUESTAR® FORENSIC latent bloodstain reagent is extremely sensitive. Blood is detected on any background: material, animal or vegetable, in liquid or solid form. Blood may be detected either pure or diluted, while fresh, or long after it has been deposited (dried or even deteriorated).

Furthermore, it allows for viewing the blood traces even if they have been rinsed away, or cleaned away (even or minute blood amounts).

The BLUESTAR® FORENSIC latent bloodstain reagent produces such an exceptionally bright chemiluminescence that complete darkness is not required for use, and no special photographic equipment is necessary to record the evidence. Standard 35 mm cameras can be used with perfect results.

The BLUESTAR® FORENSIC latent bloodstain reagent is prepared within minutes. It is then applied lightly, in a mist with a hand-held fine-spray bottle.

The blue chemiluminescence produced by the BLUESTAR FORENSIC latent bloodstain reagent is still visible several minutes after the bloodstains have been treated.

To facilitate crime scene investigation, BLUESTAR® FORENSIC can be applied numerous times over the same bloodstain without compromising later DNA typing.

B - User's Manual

1 - MIXING INSTRUCTIONS

Box content

Each BLUESTAR® FORENSIC TABLETS box holds 2 tube containing 8 pairs of tablets to make 8 x 125 ml (8 x 4 fl oz) of BLUESTAR® FORENSIC chemiluminescent solution or 1 liter (32 fl. oz) if all pairs are used together.

Required items

Prior to mixing the product, you will need the following items:

- distilled water
- spray bottle (mister) equipped with an adjustable spray nozzle

Active life

Best results are obtained when the product is used within 3 hours after mixing the tablets in water. There is therefore no requirement to rush the investigation due to immediate product deterioration.

Covered area

A 125 ml (4 fl. oz) dose is generally sufficient to investigate a 25 m² (250 sq. ft.) area, to search a vehicle or an object (clothes, rug, knife, etc.). A 1 liter (32 fl. oz) dose is generally sufficient to investigate about a 200 m² (2,000 sq. ft.) area.

Mixing procedure

1. Open the spray bottle; add 125 ml (4 fl. oz) of distilled water.
2. Take a white tablet out of the white-top tube and close the tube immediately. Take a beige tablet out of the orange-top tube and close the tube immediately. DO NOT switch caps. The white cap goes on the white-top tube and the orange cap on the orange-top tube.
3. Add the pair of tablets to the distilled water. If you need more working solution, use 125 ml (4 fl. oz) per pair of tablets.
4. Twist the head with its plunger onto the spray bottle firmly.
5. Allow about 1 or 2 minutes for complete dissolution and mixing of the chemicals, swirling gently with a circular motion of your hand. Do NOT shake the container upside down.

2 - APPLICATION INSTRUCTIONS

Lighting conditions

The BLUESTAR® FORENSIC latent bloodstain reagent produces a very bright and long lasting blue chemiluminescence that does not require total obscurity to be visible. However, at high blood dilutions, the investigation will be much easier and the risks of missing a clue much lower, if the product is applied in total darkness.

Indoors: Close all the windows, block all outside light sources, and turn off all the lights.

Outdoors: Wait for night time, and turn off all area lighting.

in an urban environment. If necessary, screen off distant light sources, or even a very bright moon, and work facing away from parasite lights.

Wait for at least 1 minute to allow your eyes to adjust to darkness. Once your pupils have dilated, you will be able to better observe the BLUESTAR® FORENSIC reaction.

Vaporization

In order to prevent biological contaminations of the revealed bloodstains, wear personal protective equipment: safety goggles, gloves, dust respirator, protective clothing.

Do not spray toward another person.

The BLUESTAR® FORENSIC latent bloodstain reagent is designed to be vaporized from waist height in a fine mist. Adjust the spray nozzle to obtain the finest mist possible. Very little of it is actually needed. Over-spraying does NOT result in improved blood detection, and in case the DNA is only available in very low quantities, over-spraying might dilute it too much for collecting exploitable samples, thus compromising its analysis.

Check how the product reacts by spraying BLUESTAR® FORENSIC on a testing sample. This test will also help you become familiar with the reaction on blood.

Spray lightly, horizontally ahead of you, at least 50 cm (2') away from the target, in a side to side sweeping motion, NOT pointing toward the ground.

Indoors: Be attentive not to saturate walls and vertical surfaces in order not to create drippings (as if you were spray painting).

Outdoors: Pay attention to wind direction, if any. Do not spray into the wind, but use it to carry a light cloud of product over the area being searched.

Identifying "false" reactions

When reacting to blood, the BLUESTAR® FORENSIC latent bloodstain reagent emits an intense light-blue chemiluminescence in the 420 to 440 nanometer range. However, "false" reactions may occur due to the presence of certain household detergents, chlorine, some paints and varnishes, copper, certain iron metabolizing plants such as lichens, thyme and some tree mosses, and certain soils containing iron.

Such "false" reactions are easily identifiable by the trained technician because their color, brightness, and duration differ from those of the typical reaction on blood. Typically, "false" reactions are markedly dimmer and whiter.

"False" reactions due to chlorinated detergents are often interesting, because they may reveal attempts to wash or clean bloodstains, and to conceal a homicide.

3 - PHOTOGRAPHY

Photography of latent blood prints developed with chemiluminescence is not fundamentally different from regular day-light photography. The same four basic elements of photography (subject lighting, film sensitivity, aperture opening, and shutter speed) interact in exactly the same way.

Photography of BLUESTAR® FORENSIC detected blood prints is easy and produces excellent results.

Equipment

The chemiluminescence produced by the reaction of the BLUESTAR® FORENSIC latent bloodstain reagent to blood is bright enough that no special equipment is needed. However, relatively long exposures may be needed for maximum picture quality and a tripod and flexible cable release are highly recommended. This will ensure the camera is motionless during the time of the exposure. A 24 mm lens is recommended.

Film

No special film is required, but it is advisable to use low-light fast films in order to obtain acceptably short exposure times. A film speed of ASA 400 is generally suitable.

Lighting conditions

Total darkness is not required. Natural low intensity diffused light is preferred. Artificial light (tungsten or fluorescent) produces yellowish or greenish pictures. A direct flash should be avoided.

Instructions to obtain good pictures

1. Set the camera on a tripod, perpendicular to the area being photographed.
2. Disable the automatic flash and the autofocus mode, if the camera has one.
3. Use a large lens aperture, typically a f/2.8 "f/stop" value.
4. Set the exposure time to "B".
5. Focus the lens manually over a spot of light provided by a flashlight over the blood area.
6. Turn off all lights. Darkness should not be complete. Pictures shot in dimmed light will allow you to view not only the trace, but other details of the scene as well.
7. Re-spray the blood print to reactivate a bright chemiluminescence reaction.
8. Shoot several pictures using different shutter speeds, typically 30 seconds.

4 - COLLECTING BIOLOGICAL PRINTS

Samples of the revealed biological prints for subsequent DNA analysis are collected using the same methods as for any kind of biological prints.

5 - STORAGE, CLEANING & DISPOSAL

Storage

The BLUESTAR® FORENSIC TABLETS latent bloodstain reagent has a 3 year shelf life AFTER MANUFACTURING (expiration date imprinted on each cardboard box).

Be certain to close the tubes immediately with their respective caps after each opening. If you wish to use the product after the expiration date, we recommend that you perform a test to check the product effectiveness.

Note: The product is warranted for 2 years after the DATE OF PURCHASE.

DO NOT attempt to store the product AFTER MIXING the tablets with water. The mixed product is an active chemical compound that oxidizes. Inert gases are constantly released and will, in time, accumulate under pressure in a sealed container, causing swelling and leaking.

Cleaning

Since the BLUESTAR® FORENSIC latent bloodstain reagent is designed to be used on presumed blood on crime scenes, all precautions and regulations related to the biohazards of blood apply when cleaning.

Disposal

Dispose of unused mix in a sink under running water. Dispose of cleaning residues according to local, state, and federal regulations applying to the biohazards of blood.

6 - MATERIAL SAFETY DATA SHEETS

Material safety data sheets (MSDS) for the BLUESTAR® FORENSIC tablets and working solution are available in PDF format on our web site:

www.bluestar-forensic.com/gb/download.php

BLUESTAR

16 Avenue de la Costa - P.O. box 246
Monte Carlo - 98000 MONACO
Tel. (+377) 97 97 31 77 - Fax (+377) 97 97 31 61

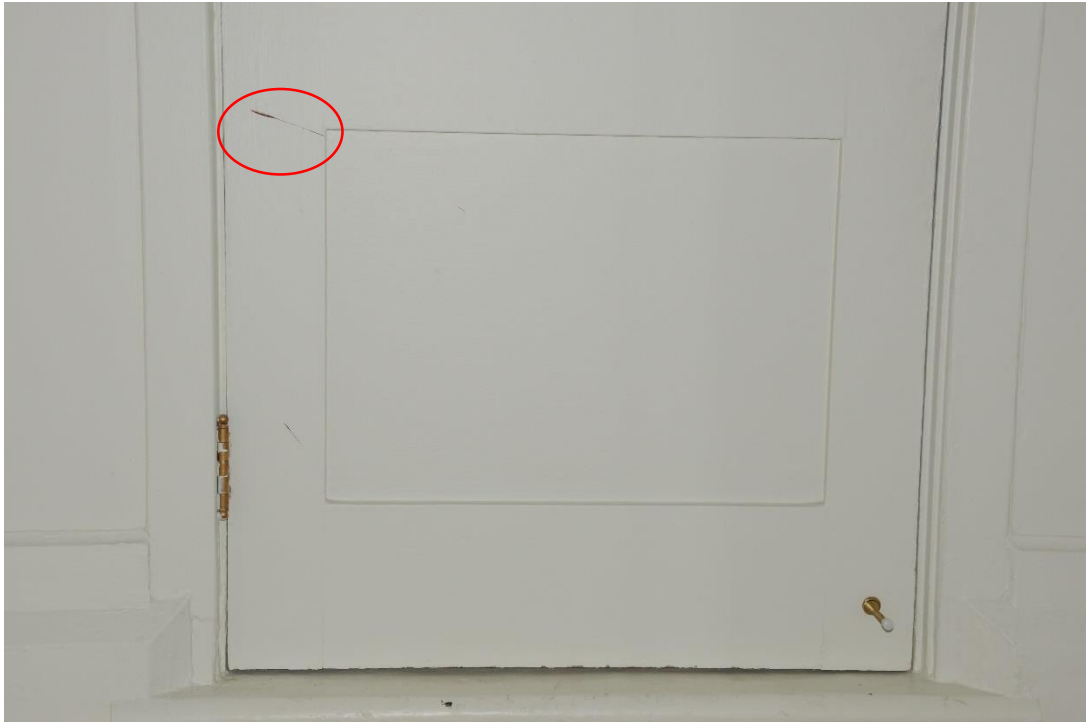
E-mail: info@bluestar-forensic.com

www.bluestar-forensic.com

Update 08/30/2006 - © BLUESTAR® 2006

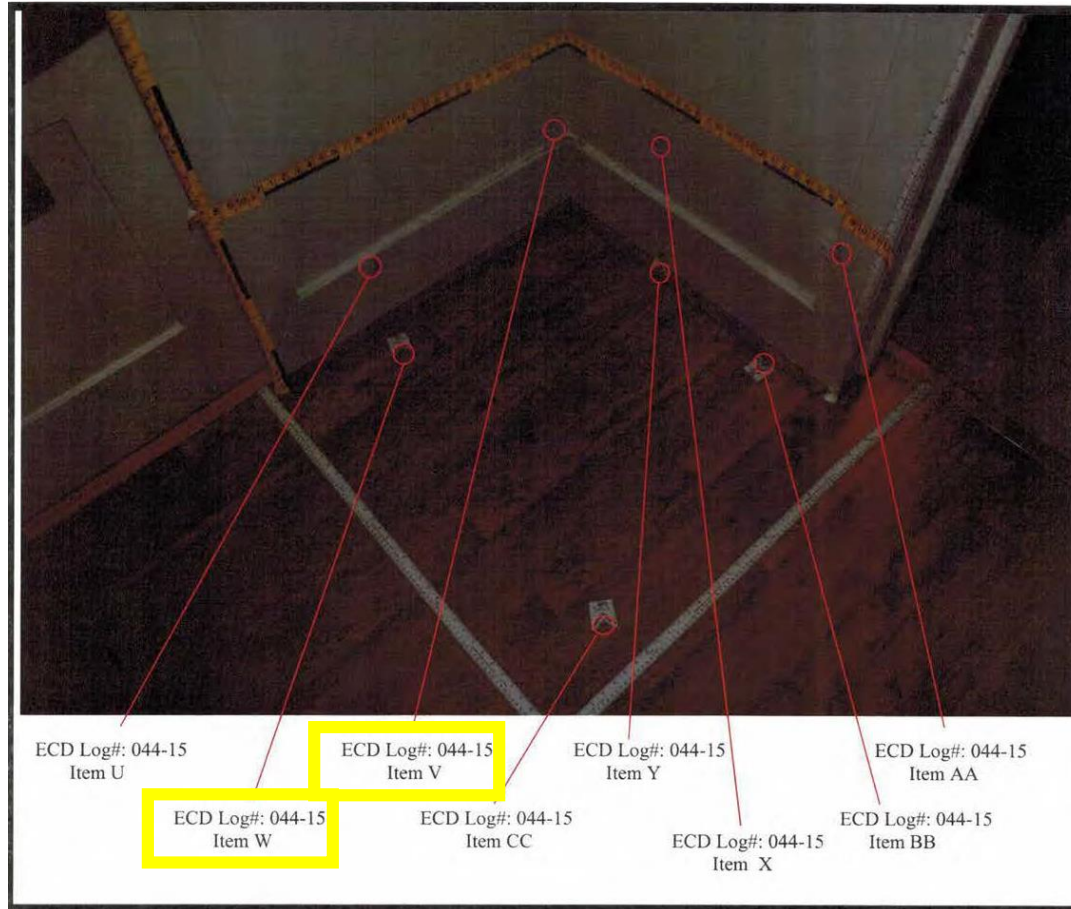
22JAN15- Visible blood was located and photographed on door. Circle indicates blood stain which was swabbed. This was V/TUR's DNA.

22JAN15- Bluestar enhancement was subsequently conducted on the door. The positive reaction was photographed.



22JAN15- Visible blood was located and photographed on floor and wall underneath the butcher block table. Swab W (floor) and Swab V (wall) were tested. This was V/TUR's DNA.

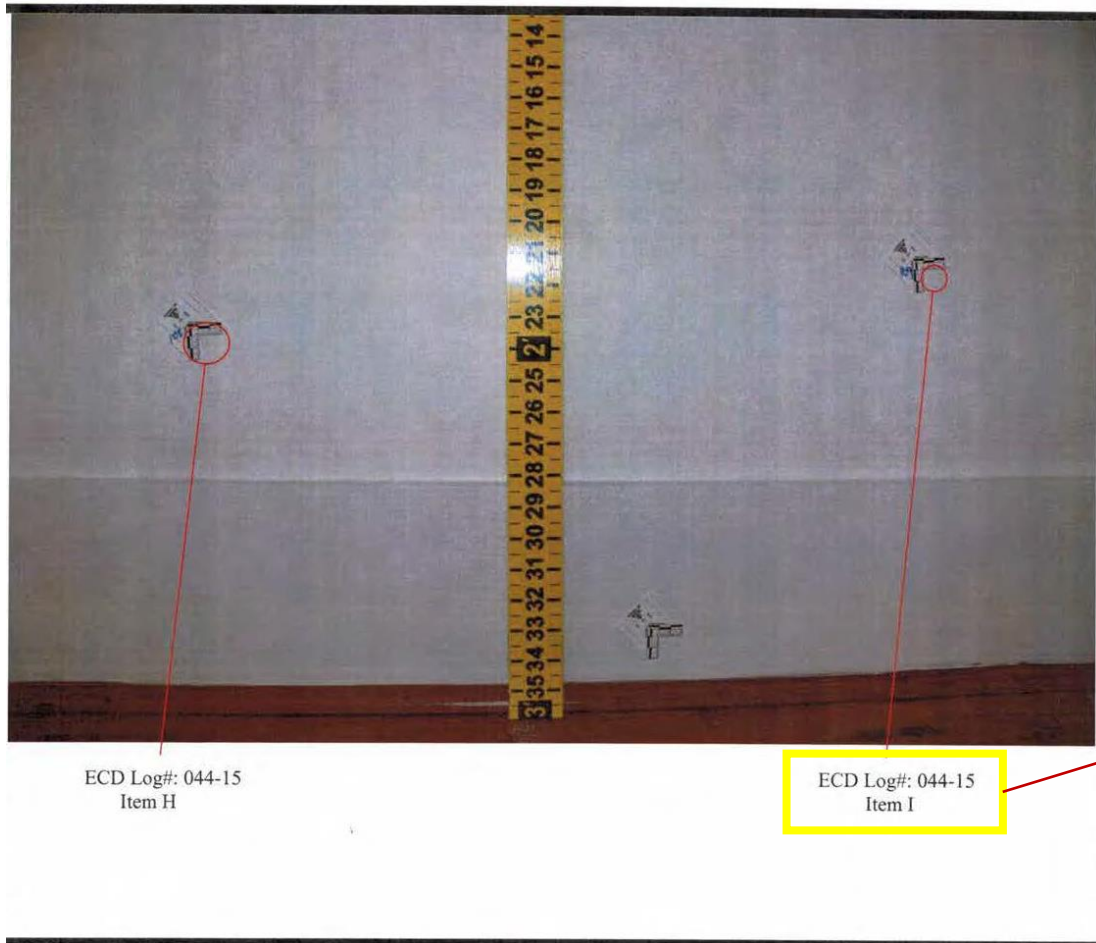
22JAN15- Bluestar enhancement was subsequently conducted on the floor. The positive reaction was photographed.





22JAN15- Visible blood was located and photographed on wall between the black shelves. Swab I was tested. This was V/TUR's DNA.

22JAN15- Bluestar enhancement was subsequently conducted on the floor, wall, and shelves. The positive reaction was photographed.



Naval Criminal Investigative Service
 CCN: 11JAN15-SEGT-0005-7HNA
 S/NETTLETON, John Robert/CAPT USN
 Bloodstain Documentation of S/NETTLETON Residence
 Date: 22Mar16

9.1 Figure 1 is a Google Earth image depicting the NETTLETON residence, CO's Dock and Bayview Club. Figures 2 and 3 show the walkway from the CO's driveway leading south, down to the CO's Dock. The stairway began at the driveway of the CO's residence and ended attached to the CO's Dock.

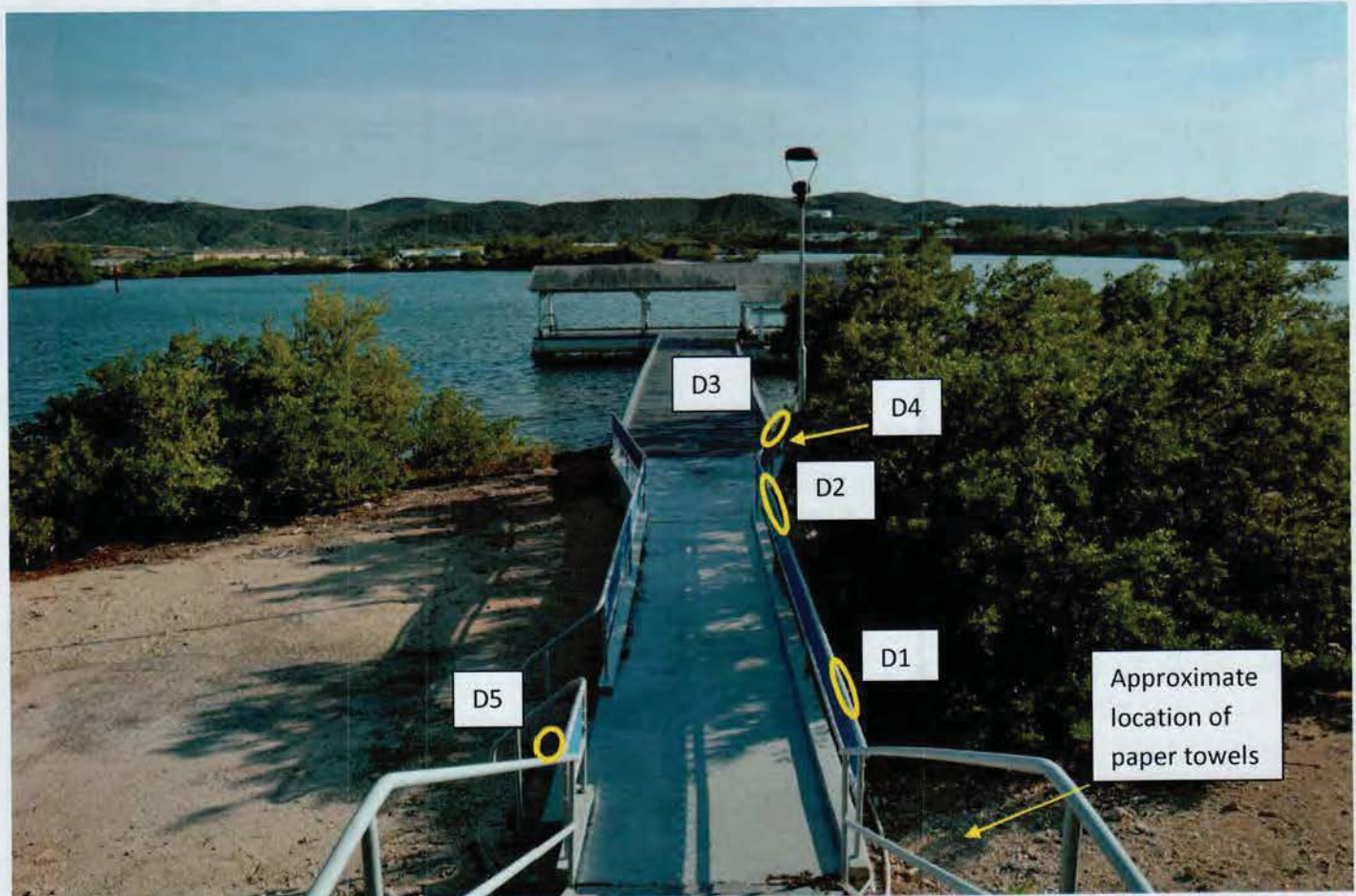


Figure 4: Overall view of the CO's Dock from halfway down the stairway facing south (DSC_2883). Labels, outlines and arrows were added to show the approximate location of items seized as evidence.

9.2 Figure 4 shows the overall view of the CO's Dock and walkway. The areas marked as D1-D5 were labeled as such for this report. These areas were swabbed subsequent to treatment with **Bluestar**⁵ and observed **luminescence**⁶ at those locations. The approximate location where **bloodstained** paper towels were previously located is noted above. There were several steps that led east off the walkway to a sandy/dirt path, which led back to the parking lot of the Bayview Club, which include the area marked as D5 in Figure 4 above.

⁵ Bluestar is designed to reveal blood, fresh, dried or still humid, washed away blood, pure or diluted, in large or minute quantity. It will create a positive reaction when in contact with iron within the heme nucleus of blood hemoglobin. It can react with other materials, such as household detergents, bleach or copper, Bluestar website.

⁶ Luminescence: the low-temperature emission of light (as by a chemical or physiological process), Merriam-Webster.

Naval Criminal Investigative Service
CCN: 11JAN15-SEGT-0005-7HNA
S/NETTLETON, John Robert/CAPT USN
Bloodstain Documentation of S/NETTLETON Residence
Date: 22Mar16

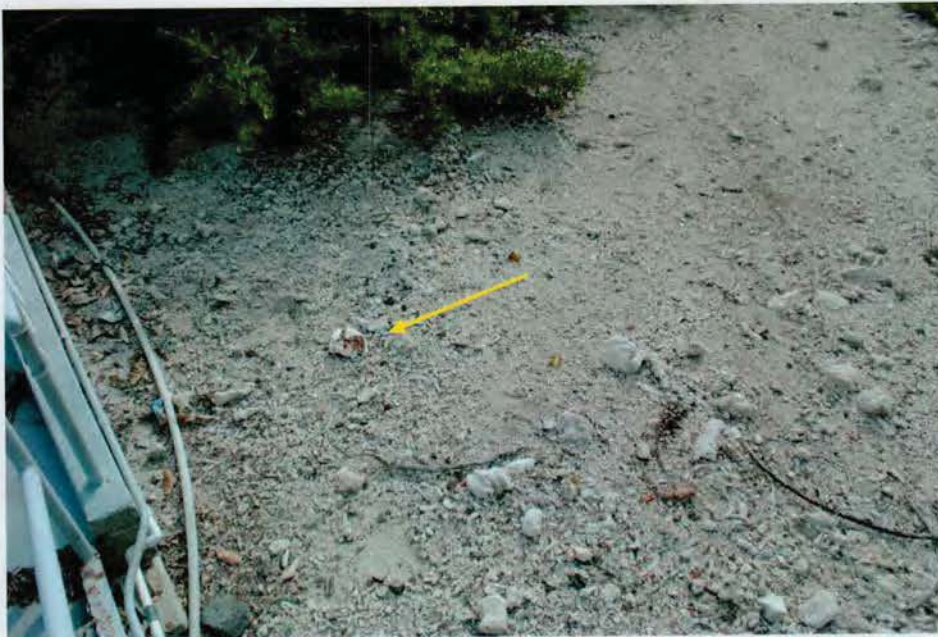


Figure 5: Midrange photograph of paper towels containing V/TUR's blood found west of the walkway leading to the CO's Dock (DSC_0068). The arrow was added to show the location of the paper towels.



Figure 6: Close-up photograph of paper towels containing V/TUR's blood found west of the walkway leading to the CO's Dock (DSC_0070).

9.3 Figures 5 and 6 above depict the stained paper towels that were located west of the walkway leading to the dock subsequent to V/TUR's disappearance. The paper towels were determined by USACIL to contain V/TUR's blood.

Naval Criminal Investigative Service
CCN: 11JAN15-SEGT-0005-7HNA
S/NETTLETON, John Robert/CAPT USN
Bloodstain Documentation of S/NETTLETON Residence
Date: 22Mar16



Figure 7: Midrange view of the west railing of the CO's Dock at the bottom of the stairway facing west (DSC_2874). The circle was added to depict D1.

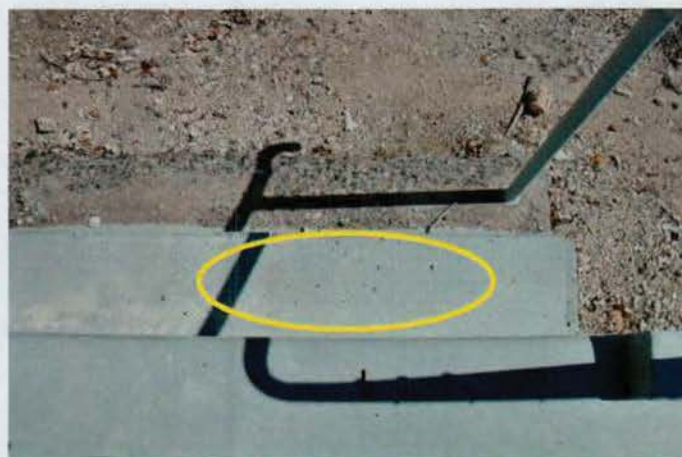


Figure 8: Midrange view of the west railing of the CO's Dock halfway down the walkway to the dock facing west (DSC_2876). The circle was added to depict D2.

Naval Criminal Investigative Service
CCN: 11JAN15-SEGT-0005-7HNA
S/NETTLETON, John Robert/CAPT USN
Bloodstain Documentation of S/NETTLETON Residence
Date: 22Mar16



Figure 9: Midrange view of the west railing of the CO's Dock near the end walkway to the dock facing west (DSC_2877). The circles were added to depict the locations of D3 and D4.



Figures 10 and 11: Midrange photograph of steps east of the walkway leading to the CO's (DSC_2879) and (DSC_2880). The circles were added to depict the location of D5.

Naval Criminal Investigative Service
CCN: 11JAN15-SEGT-0005-7HNA
S/NETTLETON, John Robert/CAPT USN
Bloodstain Documentation of S/NETTLETON Residence
Date: 22Mar16

9.4 **Bluestar** was utilized on the dirt/rock area where the paper towels depicted in Figures 5-6 were recovered. The handrail near where the paper towels were located, the curb area of the walkway at the south end of the handrail and the walkway from the end of the stairway to the dock were also sprayed with **Bluestar**. It was also used on the steps leading east and the dirt/sand area just beyond the steps. The center and west sections of the dock and several areas of the NETTLETON's boat were also sprayed with **Bluestar**. The following locations were observed to **luminesce** during the two occasions **Bluestar** was applied: an area 3'8" south of the west railing elbow (where the stairway ended and the walkway began – labeled as D1) [Figure 7], an area 23' 11" south of the west railing elbow – labeled as D2) [Figure 8], an area at the south end of the west railing (labeled as D3) [Figure 9], an area on the curb below the fold-down stairs at the end of the west railing [Figure 9] (labeled as D4), an area on the middle step leading east off of the walkway [Figures 10 and 11] (labeled as D5), and several locations on the dock and boat.

9.5 Samples were collected from the areas on the handrail, curb and step that had been observed to **luminesce**. Areas on the NETTLETON's boat and the center and west sections of the dock were presumptively tested for blood utilizing **Hexagon OBTI**⁷ with no positive results.

9.6 According to laboratory testing conducted by USACIL, no blood was detected on D1. A chemical indication of blood was obtained on the swab from the curb (D4) but no confirmatory test was conducted due to the limited sample. No DNA was detected. A chemical indication of blood was also obtained from the steps (D5); however, the presence of blood could not be confirmed with immunological testing. The partial DNA profile obtained from the swab from the steps was consistent with the DNA profile of V/TUR. The DNA profile obtained from a swabbing of the stain on the paper towel matched the DNA profile from V/TUR.

⁷ Hexagon OBTI will create a positive reaction when in the presence of human hemoglobin, Hexagon OBTI User Manual.

TECHNICAL NOTE

Shanan S. Tobe,¹ M.Sc.; Nigel Watson,¹ Ph.D.; and Niamh Nic Daeid,¹ Ph.D.

Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular-Weight DNA

ABSTRACT: Luminol, leuchomalachite green, phenolphthalein, Hemastix[®], Hemident[™], and Bluestar[®] are all used as presumptive tests for blood. In this study, the tests were subjected to dilute blood (from 1:10,000 to 1:10,000,000), many common household substance, and chemicals. Samples were tested for DNA to determine whether the presumptive tests damaged or destroyed DNA. The DNA loci tested were D2S1338 and D19S433. Leuchomalachite green had a sensitivity of 1:10,000, while the remaining tests were able to detect blood to a dilution of 1:100,000. Substances tested include saliva, semen, potato, tomato, tomato sauce, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride. Of all the substances tested, not one of the household items reacted with every test; however, the chemicals did. DNA was recovered and amplified from luminol, phenolphthalein, Hemastix[®], and Bluestar[®], but not from leuchomalachite green or Hemident[™].

KEYWORDS: forensic science, luminol, leuchomalachite green, phenolphthalein, Hemastix[®], Hemident[™], Bluestar[®], presumptive tests, sensitivity, specificity, DNA recovery

Blood is the most common and perhaps the most important form of evidence in the world of criminal justice today (1). Blood evidence associated with a crime can provide essential information that may help solve a case, collaborate witness testimony, define a scene of crime, link a suspect and scene, or simply point the investigation in a new direction (1,2). Therefore, it is important to identify any stain that could potentially be blood at a crime scene. Obvious bloodstains should never be contaminated with any reagent (3). When encountered with a potential bloodstain that cannot be identified immediately, several questions enter the mind of an investigator or forensic scientist. These include "What is that stain?"; "Could it be blood?"; or if a stain is expected or suspected, and is absent, "Was there blood here at one time?" Cox (4) describes the attributes that a good presumptive test for blood should have: it should be sensitive, specific, quick, simple, and safe.

More recently, these questions have expanded to include "Whose blood is this?"; "Can it be excluded from a control or known sample?"; and "Is there enough genetic material here to obtain a complete DNA profile?" It is therefore obvious that within a forensic context, the most important components of blood are those that can be used for blood identification and to individualize it (5).

In order for these presumptive tests for blood to function properly, they must detect a component of blood, which ideally should not be commonly found in the everyday environment. Therefore, most presumptive tests for blood rely on the peroxidase-like ac-

tivity of hemoglobin. This is a component of blood that is not commonly found in the everyday environment, although there are other substances found in fruits and vegetables that perform a similar function.

In the past half-century, several studies have been conducted on the sensitivity and specificity of presumptive tests for blood, and their effect on subsequent DNA analysis.

In the past 50 years, there have been many tests conducted on the sensitivity of presumptive blood tests (4,6–15). The findings of these studies are in great contradiction with each other. Sensitivities for luminol range from 1:200 (11) to 1:100,000,000 (6); from 1:200 (11) to 1:100,000 for leuchomalachite green (LMG) (8); and from 1:2,000 (12,13) to 1:10,000,000 for phenolphthalein (9). The various differences in the sensitivities reported by different researchers of presumptive blood tests are probably caused by differences in reagent concentrations, methods of preparation of samples, reagents and results, and in the type of material containing the blood (4). Grodsky et al. (8) also add that dried bloodstains are not comparable with the same amount of blood dissolved in a solution. They further add that many of the discrepancies observed are probably due to the presumptive test reagents being added directly to a dilute blood solution, thereby also diluting the reagent, while in other cases the dilute blood solutions are dried first and then tested with full-strength reagents (8).

In the past half century, many tests have been conducted on the specificity of presumptive blood tests. These tests for specificity include changing substrates, adding material and chemicals to the bloodstains, and testing to see whether the reagents will react with substances other than blood (4,7,11,13,14,16–21). Grodsky et al. (8) believe that studies involving the various presumptive blood tests indicate that there is a degree of interference with some of them that effectively prevents their effective use as a test for the

¹Department of Pure and Applied Chemistry, Centre for Forensic Science, Strathclyde University, 204 George Street, Glasgow G1 1XW, U.K.

Received 5 Feb. 2006; and in revised form 12 July 2006 and 2 Aug. 2006; accepted 1 Sept. 2006; published 8 Dec. 2006.

presumptive identification of blood. Therefore, this must be addressed and examined with experimentation.

The ideal presumptive blood test is one that is specific to blood (more specifically to human blood), has a high sensitivity, will meet the Frye standard, and will not damage underlying DNA so that a full DNA profile can be obtained after the reagent's use (5,22). New reagents will be tested with the ones most commonly used by police and forensic scientists throughout the world: Kastle-Meyer (KM), leuchomalachite green, and luminol (23,24). The ease of transport, ease of use, working life, and storage will be determined and discussed for the three new reagents.

Current literature reports differing sensitivities for the various blood detection tests, often conflicting in their results. Therefore, the sensitivity limits of the reagents will be tested and the limits will be determined.

The specificity of the new reagents will be tested with substances commonly known to interfere with traditional reagents, or those that could be mistaken for blood spatter in some situations.

DNA will be collected and PCR performed to determine whether the reagents have limits less than, equal to, or exceeding that of current DNA detection techniques.

Materials and Methods

Samples

Blood samples were taken from an anonymous donor. All equipment used to extract, store, apply, and manipulate the blood for the experiments was sterile. The equipment was either open from sterile packaging or autoclaved at 120°C for 20 min.

Blood from the donor was used for all experiments and for positive controls. The blood was extracted by creating a small lancet wound in the finger of the donor and was not subject to any form of anticoagulants or other contaminants.

Reagents

Luminol (3-aminophthalhydrazidem), LMG, and phenolphthalein KM were prepared according to Strathclyde University, Centre For Forensic Science guidelines. Hemastix[®] (instructions included with reagent), Hemident[™] (MacPhails[™] Reagent; instructions included with reagent), and Bluestar[®] (instructions included with reagent) were from commercially available kits provided by WA Products (Essex, U.K.) (product codes: B23014, B23013, B23014). All reagents were used according to the manufacturer's guidelines.

Positive controls were taken by applying the reagent to a blood-stained piece of filter paper. Negative controls were performed by applying the reagents to a fresh piece of filter paper with no trace of blood. The positive control was retained for further DNA testing.

Sensitivity Testing

Autoclaved bottles (125°C for 20 min) and distilled H₂O were used. Water was measured using a graduated cylinder and blood was added using a Gilson pipette. Differing low concentrations of blood were achieved by making a stock solution of blood and distilled water. Solutions of 1:10,000; 1:100,000; 1:1,000,000; 1:5,000,000; and 1:10,000,000 were prepared.

A set of 25 1 cm × 1 cm pieces of filter paper were placed in each of the diluted blood solutions for each of the presumptive reagents tested. The pieces of filter paper were then removed and allowed to dry for 72 h. Each of the pieces of filter paper was then

tested with its corresponding reagent to see whether the blood present was detectable. The reagents were added directly to the 1 cm² pieces of filter paper. The time taken for the reagent to register a positive result was determined and recorded. Tests were considered negative if reagents failed to react within 4 min of exposure to the blood-stained filter paper. The treated pieces of filter paper that had not reacted with any reagents were retained for subsequent DNA analysis.

Specificity Testing

Substances found to give false positives previously as reported by other authors, or substances which could be mistaken for blood, were tested. The tests were also carried out on saliva and semen.

The six different reagents were tested against saliva, semen, potato, tomato, tomato sauce, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride.

For each of the presumptive reagents tested, a large piece of filter paper (approximately 100 cm²) was exposed to each of the substances being tested in 25 separate sample stains. These were allowed to dry for a minimum of 18 h. Each of the pieces of filter paper, and subsequent stains, were then tested with their corresponding reagent to see whether the substance caused a reaction. The time taken for the reagent to register a positive result was determined and recorded. Tests were considered positive if there was any color change, and were considered negative if there was no observable color change within 4 min of exposure to the stained filter paper.

DNA Testing

The Chelex method of DNA purification and recovery was used. The protocol consisted of sterile distilled water, 20% Chelex suspension, and extraction buffer. For each sample, 0.5 mL Sterile H₂O was pipetted to a 0.5 mL Eppendorf tube. A small 3 mm² section of the positive control (which had been exposed to the reagents) was added to the tube. For the sensitivity testing, the entire 1 cm × 1 cm section of the filter paper was added to the tube. For the controls, a 3 mm² section of bloodstain was placed in the tube.

All the samples were incubated at room temperature for 25 min with occasional inverting. They were then centrifuged at maximum for 2 min. Each tube had 0.35 mL of the supernatant removed and then the pellet was resuspended. Fifty microliters of 20% Chelex was added to each tube and they were then incubated at 56°C for 30 min. Samples were then vortexed for 10 sec, boiled for 10 min, and then vortexed for a further 10 sec. Samples were then centrifuged at maximum for 2 min. The supernatant was removed and retained in a separate Eppendorf tube and the pellet was discarded. The retained supernatant was stored frozen.

A full commercial DNA profiling kit will not be used as the amount of information that a full 10 or 14 loci (using SGMPlus[™] or IdentifilerPlus[™], Applied Biosystems, Foster City, CA) profile would provide is not needed in this study. Instead, two STR loci from a well-used commercial kit, SGMPlus[™], will be amplified. The STR loci to be used are D19S433 and D2S1338, the smallest and largest loci, respectively (25). This will allow for both ends of the spectrum to be amplified, as larger products are more likely to drop out in degraded DNA than smaller loci are. Therefore, if only D19S433 amplifies and D2S1338 drops out, it would mean that partial amplification could likely be obtained from a commercial kit. If both D19S433 and D2S1338 amplify, then this should

indicate that a commercial STR typing kit would be able to obtain a full profile off the samples.

As the exact primer sequence used by Applied Biosystems is not known, different primers were used. The primer sequences were obtained from UniSTS (26), which is a comprehensive database of sequence-tagged sites (STSs) defined by PCR primer pairs and are associated with additional information such as genomic position, genes, and sequences (26). The primer information for the two loci was given as:

D19S433

Forward: 5'-HEX-CCTGGGCAACAGAATAAGAT-3'

Reverse: 5'-TAGGTTTTTAAGGAACAGGTGG-3'

D2S1338

Forward: 5'-HEX-CCAGTGGATTTGGAAACAGA-3'

Reverse: 5'-ACCTAGCATGGTACCTGCAG-3'.

Primer sets for D19S433 and D2S1338 were each run in a separate PCR of 25 μ L total; 2 μ L of each primer was used and 5 μ L of template DNA. PCR was performed on a Perkin Elmer GeneAmp PCR System 2400 (Boston, MA). Thirty two cycles of 94°C for 30 sec; 55°C for 30 sec; 72°C for 1 min and 30 sec; and a final extension of 45 min at 72°C were performed.

Eleven experimental samples were run. Six correspond to all of the positive controls, 3 were from the dilution sets 1:10,000, 1:100,000, and 1:1,000,000, a positive control from the blood donor, and a negative control.

An ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) was used to analyze all samples.

Statistical Tests

The test used to compare the different reagents was the χ^2 test for consistency in a $2 \times K$ table.

Results and Discussion

The positive control of luminol reacted instantly with blood, giving a blue luminescence appearing at the site of the deposition; this persisted for over 1 min. The negative control did not react on addition of the reagent. Grodsky et al. (8) believe that luminol's only serious disadvantage, other than interference, is its requirement of near or complete darkness in order to perceive the chemiluminescence.

The LMG-positive control reacted within a few seconds of application of the H₂O₂, with a blue/green color appearing at the site of blood deposition. The negative control did not react on addition of the H₂O₂ but if left out will develop a green ring around where reagents were deposited.

The phenolphthalein KM positive control reacted within a few seconds of application of the H₂O₂, with a pink color appearing at the site of blood deposition. The negative control did not react on addition of the H₂O₂; however, there was a reaction after several minutes (greater than the 4 min timed) with a pink color developing around the edges of the area of reagent deposition.

The Hemastix® reagent strips-positive control reacted instantly on application to the blood by turning dark gray/green; the site where the reagent strip touched the filter paper also turned dark green/blue where there was blood. The negative control did not react on addition of the H₂O and there was no reaction on the filter paper; however, there was a reaction after several minutes (greater than the 4 min timed), with the reagent pad turning light green.

The ease of transport and use of the Hemastix® reagent strips is excellent. The strips are easily stored and transported and there is no risk of chemical spills or solution breakdown or contamination, all that is required is some distilled water (tap water would most likely also be fine) and a desiccant (provided with the strips) with the reagent strips. They are easy to use, and easy to transport. There is a range of color reactions to compare with on the container, for accurate reading of the strips. According to the manufacturer, storage is provided in the container and has a life of 6 months from initial opening, and about a year if unopened.

The Hemident™ (MacPhail's reagent)-positive control reacted within a few seconds of application of the H₂O₂, with a dark green/blue color appearing at the site of blood deposition. The negative control did not react on addition of the H₂O₂ but if left out will develop a green ring around where reagents were deposited.

If used according to the manufacturer's guidelines, the Hemident™ test is easy to transport and use. The ampoules are not easily broken, and the case provides a convenient disposal vessel. There are no instructions for any special storage conditions, or any expiry date indicated.

The Bluestar®-positive control reacted instantly, with a blue luminescence appearing at the site of blood deposition; this dissipated within 30 sec. The negative control did not react on addition of the reagent.

The reagent was easy to prepare from the two tablets, which were mixed directly together into a spray bottle with water (tap water can be used). The tablets can be brought to a scene separately, so there is no risk of leaking bottles of reagents. Bluestar® was extremely easy to use and was effective by just spraying over an entire area for full coverage. The working life of the solution is a problem as it is quite short once the solution is mixed and may only be reactive for a few hours. The tablets come in two separate foil-wrapped packages, but a warning is given that the product is stored under pressure and it should not be stored in the home or car without proper precautions according to the manufacturer. There is no expiry date given with the tablets, indicating that they are stable if stored with the proper precautions. The only problem, much like luminol, is that the product requires complete or near-complete darkness to be effective.

Sensitivity

The approximate numbers of erythrocytes, leukocytes, and hemoglobin molecules, as given by Marieb (27), were calculated for each of the five dilution factors and are shown in Table 1. Table 2 illustrates the results obtained for the sensitivity portion of the experiment.

The luminol reagent reacted instantly, with both the 1:10,000 and 1:100,000 dilution factors producing a blue luminescence. The luminescence lasted for close to a minute. However, both dilution factors were much less intense than the positive control of whole blood. The reaction with the 1:100,000 dilution factor was extremely faint. There was no reaction with dilutions of 1:1,000,000, 1:5,000,000, or 1:10,000,000 within the 4 min of timed experimentation.

LMG reacted at a dilution factor of 1:10,000. All samples except one showed a positive reaction within 1 min. The samples turned a green color within 1 sec of the application of the LMG reagent and the H₂O₂. A single sample did not show a positive reaction within the 4 min of timed experimentation. The LMG reagent did not show a positive reaction at dilution factors of 1:100,000, 1:1,000,000, 1:5,000,000, or 1:10,000,000 within the 4 min of timed experimentation.

TABLE 1—Distribution of blood cells for the different dilution factors, calculated from the values given in Marieb (27).

Blood 1 mL = 1 mm ³	Erythrocytes		Hemoglobin		Leukocytes	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1:1	4,300,000	5,200,000	1.075E+15	1.3E+15	4000	11,000
10:1	43,000,000	52,000,000	1.075E+16	1.3E+16	40,000	110,000
1:10,000	430	520	1.075E+11	1.3E+11	0.4	1.1
1:100,000	43	52	10,750,000,000	13,000,000,000	0.04	0.11
1:1,000,000	4.3	5.2	1,075,000,000	1,300,000,000	0.004	0.011
1:5,000,000	0.86	1.04	215,000,000	260,000,000	0.0008	0.0022
1:10,000,000	0.43	0.52	107,500,000	130,000,000	0.0004	0.0011

The phenolphthalein reagent registered a positive reaction for all samples at a dilution factor of 1:10,000. The samples turned pink after 45 sec of the introduction of the reagent and H₂O₂. At a dilution factor of 1:100,000, three of 25 samples showed a positive reaction: two of them at 1 min and 30 sec, and the third at 2 min and 30 sec. The phenolphthalein reagent did not show any reaction with dilution factors of 1:1,000,000, 1:5,000,000, or 1:10,000,000 within the 4 min of timed experimentation.

The Hemastix[®] reagent strips reacted with the 1:10,000 dilution by first causing a color reaction with the filter paper. The filter paper changed to a green color where the Hemastix[®] was pressed within a few seconds. The actual Hemastix[®] took between 30 and 60 sec to register a reaction. Eighteen Hemastix[®] were positive for +25 erythrocytes; the remaining seven registered positive for +80 erythrocytes.

The Hemastix[®] reagent strips reacted with the 1:100,000 dilution by first causing a color reaction with the filter paper. At 1 min, one of the samples showed a color change on the filter paper of a green color. The rest of the samples showed this same reaction at between 1 min and 45 sec and 2 min. At 3 min and 45 sec, 17 of the reagent strips were a very light shade of green, corresponding with a trace 10 hemolyzed sample according to the instructions. Four of the strips registered +25 erythrocytes at the same time. The remaining four strips registered a negative result at 4 min.

The Hemastix[®] reagent strips did not react with the 1:1,000,000, 1:5,000,000, and 1:10,000,000 dilutions. There was no color change on the filter paper or on the reagent strips.

There were no previous literature values for the sensitivity of Hemastix[®] although the package claims to be able to detect blood in urine down to 10 erythrocytes, which equates to between a 1:100,000 and 1:1,000,000 dilution factor (Table 1). This is consistent with the results obtained in this experiment, although the strips should be read at 60 sec and a reaction was not observed on the strips until between 3 and 4 min after initial application to the stain.

The Hemident[™] reagent reacted with most of the samples at the 1:10,000 dilution. One sample reacted before the addition of

the H₂O₂ with a color change to green; two other samples did not register a reaction. The samples that did react showed a green/blue color change at the edges of the filter paper, predominantly in the corners, which occurred within 1 min of addition of the H₂O₂.

The Hemident[™] reagent did not react with most of the samples at the 1:100,000 dilution. Two of the samples showed a positive result at 4 min. The remainder did not show any reaction. There was no reaction with the samples diluted to 1:1,000,000, 1:5,000,000, or 1:10,000,000 within the 4 min of timed experimentation.

There were no previous literature values for the sensitivity of Hemident[™], but the package claims a capability of identifying one part per million of blood (28). The findings of this study do not confirm this. Hemident[™] is slightly more sensitive than leuchomalachite green, but does not even approach the sensitivity it claims to have. Two samples showed a positive reaction at 1:100,000 dilution, which is 10 times more sensitive than leuchomalachite green, but this was not consistent over all 25 samples, and it is still one-tenth of the sensitivity claimed.

The Bluestar[®] reagent reacted instantly with the 1:10,000 with a blue luminescent glow but faded within a few seconds. The 1:100,000 dilution showed slight reactivity, with five of the 25 samples showing a very faint positive, which faded in a few seconds. However, both dilution factors were much less intense than the positive control of whole blood. There was no reaction with dilutions of 1:1,000,000, 1:5,000,000, or 1:10,000,000 within the 4 min of timed experimentation.

The Bluestar[®] reagent has no previous tested sensitivities, although the company claims sensitivity to 1:1,000 dilution (29). This was not found to be consistent with this study as luminescence was detected at 1:100,000 dilution of blood in water, 100 times more sensitive than what is claimed by the company. This luminescence was faint and short-lived, but was still detectable.

Specificity

Table 3 gives the specificity results for all reagents.

TABLE 2—Sensitivity results for the six different reagents.

Dilution	Reagent					
	Luminol	LMG	KM	Hemastix [®]	Hemident [™]	Bluestar [®]
1:10,000	1	1	1	1	1	1
1:100,000	1	NR	2	2	4	1
1:1,000,000	NR	NR	NR	NR	NR	NR
1:5,000,000	NR	NR	NR	NR	NR	NR
1:10,000,000	NR	NR	NR	NR	NR	NR

The shortest reaction time is shown here.

A positive reaction was any sort of color change to the stain (or reagent strip in the case of Hemastix[®]); 0, color change before all reagents were added; 1, color change within 1 min of all reagents being added; 2, color change within 1–2 min of all reagents being added; 3, color change within 2–3 min of all reagents being added; 4, color change within 3–4 min of all reagents being added;

NR, indicates that there was no reaction within the 4 min of timed experimentation; KM, Kastle-Meyer; LMG, leuchomalachite green.

TABLE 3—Specificity results for the six different reagents.

Substance	Reagent					
	Luminol	LMG	KM	Hemastix [®]	Hemident [™]	Bluestar [®]
Saliva	NR	NR	NR	1 (3)	NR	NR
Semen	NR	NR	1 (25)	NR	0 (25, white)	NR
Potato	NR	0 (6, green)	3 (7)	1 (25)	NR	1 (25)
Tomato	NR	NR	NR	1 (23)	NR	1 (25)
Tomato sauce	NR	NR	4 (6)	NR	NR	NR
Tomato sauce w/meat	NR	NR	NR	4 (6)	NR	1 (25)
Red onion	NR	0 (5, pink)	0 (25, yellow)	1 (21)	0 (6, pink)	1 (25)
Red kidney bean	NR	NR	2 (5)	NR	NR	1 (25)
Horseradish	NR	NR	4 (25)	NR	NR	1 (25)
1 M Ascorbic acid	NR	NR	0 (25, yellow)	NR	1 (11)	1 (25)
Bleach solution 5%	NR	NR	3 (2)	NR	1 (5)	1 (25)
10% Cupric sulfate	1 (25)	0 (25, blue)	0 (25, blue)	1 (25)	0 (25, blue)	1 (25)
10% Ferric sulfate	1 (25)	0 (25, orange)	0 (25, yellow)	1 (25)	0 (25, red/brown)	1 (25)
10% Nickel chloride	1 (25)	0 (25, blue)	0 (25, green)	1 (25)	0 (25, green)	NR

The shortest reaction time is shown here. Numbers in parentheses indicate the number of samples that reacted out of 25 and the color change observed if different from that of a reaction with blood.

A positive reaction was any sort of color change to the stain (or reagent strip in the case of Hemastix[®]); 0, a color change before all reagents were added; 1, indicates a color change within 1 min of all reagents being added; 2, color change within 1–2 min of all reagents being added; 3, color change within 2–3 min of all reagents being added; 4, color change within 3–4 min of all reagents being added; NR, indicates that there was no reaction within the 4 min of timed experimentation.

There was a reaction between the luminol reagent and 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride indicated by a blue chemiluminescence. Both the 10% cupric sulfate and 10% ferric sulfate showed an immediate reaction on addition of the luminol. The 10% nickel chloride also showed a reaction on addition of the luminol; however, the intensity of this reaction was far less than for the ferric and cupric sulfates and it did not occur instantly. The reaction took several seconds to become visible.

Luminol's reaction with the metal salts was expected as Grodsky et al. (8) noted that the common substances that interfere with luminol are copper-containing surfaces.

Contrary to the literature findings, this study found that luminol only reacted with blood and the metal salts. Bleach gave no reaction, but this could be because the bleach solution was only 5% concentration, and that it was not tested right away but first allowed to dry for at least 18 h. Kent et al. (20) noted that when bleach-treated blood is left for several days, the interference by bleach is diminished. The negative reaction observed may be due to the storage time of the sample. Luminol was expected to react with the potato and horseradish as it has been used to study vegetable peroxidase reactions, such as the horseradish peroxidase reaction (30), and Albrecht noted that fresh potato juice caused luminescence (16). This could once again be due to the substances' drying time before testing.

LMG showed a reaction with several of the substances tested. However, the results of these reactions would not be mistaken for a reaction with blood. All of the substances that reacted did so after the addition of the LMG reagent but before the addition of the H₂O₂. This agrees with the findings of Alvarez de Toledo and Valero, who noted that many chemical oxidants may yield the reaction in the absence of H₂O₂ (31). Blood only reacts after the addition of the hydrogen peroxide and then only at the site of blood deposition. Therefore, none of the substances tested react in the same manner as blood and could not be mistaken for a reaction with blood.

Several substances reacted with the phenolphthalein reagent. Semen stains showed a very light pink color change at 45 sec, which grew stronger as the timing approached 2 min. Seven potato samples showed a slight pink color change within 2 min of introduction of the reagents. Six tomato sauce samples showed a pink color at 3 min and 45 sec. Red onion samples turned yellow after

the addition of the KM reagent, but before the addition of H₂O₂. The horseradish samples all showed a very slight pink color change at 3 min. The 0.1 M ascorbic acid samples turned yellow after the addition of the phenolphthalein reagent, but did not show any further color change on addition of the H₂O₂. Two of the 5% bleach samples showed a slight pink color at 2 min, but the rest did not show a color change within the 4 min of timed experimentation. On addition of the phenolphthalein reagent, the 10% cupric sulfate samples turned blue. On addition of the H₂O₂, the samples instantly turned brown and foamed. All 25 of the 10% cupric sulfate samples then developed an intense pink color around the stain, 11 within 1 min and the remaining 14 within 2 min and 30 sec. The 10% ferric sulfate samples turned yellow/brown on addition of the phenolphthalein reagent and the stains had dark edges. The samples foamed on addition of H₂O₂. At 1 min and 30 sec, 13 of the samples showed a pink color developing around the stain, and two additional samples showed this same color change at 3 min. The remaining samples did not develop any color around the stains in the 4 min of timed experimentation. The 10% nickel chloride turned light green on addition of the phenolphthalein reagent. This color deepened on addition of the H₂O₂. Eight of the samples developed a pink color around the stain at 3 min. The rest of the samples failed to react within the 4 min of timed experimentation.

The reaction of the phenolphthalein reagent with other substances differs from Pinker (32), who did not find even one substance that would give a true positive reaction with phenolphthalein. This does not correspond with the current findings as semen caused a reaction at 45 sec, that grew stronger with time. The potato stains reacted the same way, as did tomato sauce, red kidney beans, horseradish, and 5% bleach, which all reacted at some point within the 4 min of timed experimentation. This time delay in reaction is much like the time delay observed for dilute blood samples (1:100,000) and therefore, any of these stains could be conceivably mistaken for very dilute blood.

The remaining substances reacted before all the reagents were added, or did not form the pink color as an expected bloodstain would. Both the red onion and the 0.1 M ascorbic acid samples turned yellow on addition of the phenolphthalein reagents but before the H₂O₂ was added. The metal salts also all reacted before

the H₂O₂ was added by turning blue (10% cupric sulfate), yellow/brown (10% ferric sulfate), and light green (10% nickel chloride).

There was a reaction between the Hemastix[®] reagent strips and saliva, potato, tomato, tomato sauce with meat, red onion, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride. The Hemastix[®] reagent strips reacted with three of the saliva samples turning the paper green. One reacted within 1 min, and the remaining two samples reacted within 2 min. The actual reagent strips did not show a reaction within the 4 min of timed experimentation, nor did any of the remaining 22 samples. All of the potato stains produces a color reaction by turning green within 15 sec, which darkened to blue as time progressed. There was no reaction with the actual reagent strips within the 4 min of timed experimentation. The tomato samples reacted within 1 min by turning a very light green, which darkened as time passed. Two of the stains did not react, and most of the reagent pads did not react within the 4 min of timed experimentation. Two of the pads showed a very slight green color change at 4 min. Six of the tomato sauce with meat samples reacted at 3 min. The six stains turned green around the edges at 3 min. The rest of the sample stains as well as all of the reagent pads did not react within the 4 min of timed experimentation. Twenty-one of the red onion samples turned a very light green at 1 min with the color darkening to dark green/blue as time progressed. Three of the reagent pads show a green line running horizontally, the remaining pads and 4 stains did not react within the 4 min of timed experimentation. The 10% cupric sulfate samples showed an instant blue/green color on the stain. The reagent pads appear as trace (spots of green) non-hemolyzed and progress to a dark green/blue uniform color. The 10% ferric sulfate samples all turned instantly green/blue at the center of the stain, which progressed to brown and then yellow along the outer margins of the stain. The reagent pads showed a small trace (spots of green color) at 4 min. The 10% nickel chloride sample stains all turned instantly green/blue on application of the reagent. The reagent pads were all negative after the 4 min of timed experimentation except for one, which showed a green/blue color at the end of the strip.

The Hemastix[®] reagent strips were quite reactive with eight of the substances tested. All of the substances showed a green color, which may or may not have progressed to blue. This is the same reaction observed on blood samples, except that when exposed to blood the reagent strips also showed a reaction. This was not the case for saliva, potato, and the tomato sauce with meat, which did not react with the reagent pads.

The samples that did show a reaction on the actual reagent pads were tomato, red onion, 10% cupric sulfate, 10% ferric sulfate,

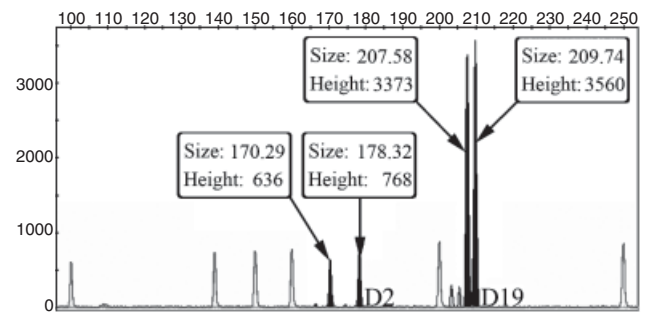


FIG. 1—Positive control DNA. Loci D2S1338 and D19S433 are shown with peak size and height.

and 10% nickel chloride. These samples showed the same reaction as dilute blood samples would, and in the case of the 10% nickel chloride samples, showed a color change indicative of whole blood.

The Hemident[™] reagents did show a color reaction with semen, red onion, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride. All of the semen stains turned white on addition of the first reagent, but there was no further reaction on addition of the H₂O₂. Six of the red onion samples turned pink on addition of the first reagent but there was no further reaction on addition of the H₂O₂ during the 4 min of timed experimentation. Two of the 0.1 M ascorbic acid samples turned instantly positive with a blue/green color. Within 30 sec, nine other samples a slightly positive, with a light blue/green color developing and increasing with intensity as time progressed. The remaining 14 0.1 M ascorbic acid samples did not react with the reagents during the timed experiment. Five of the 5% bleach samples turned light blue/green along their margins 30 sec after addition of the H₂O₂; the remaining 20 samples did not react with the reagents within the 4 min of timed experimentation. The 10% cupric sulfate samples all turned slightly blue on addition of the first reagent, and 12 developed an instant blue/green color around their edges on addition of the H₂O₂. A further five samples showed the same blue/green edges after 1 min and this color intensified with time, but no other samples reacted. The 10% ferric sulfate sample all turned brown/red on addition of the first reagent and instantly turned grass green on addition of the H₂O₂. This color darkened to blue/green over time. The 10% nickel chloride samples all turned very light green after the addition of the first reagent, but there was no further color change on addition of the H₂O₂ during the 4 min of timed experimentation.

TABLE 4—DNA results for the various presumptive tests, positive and negative controls, and dilution series.

	D2S1338		D19S433	
	Peak 1 (Height)	Peak 2 (Height)	Peak 1 (Height)	Peak 2 (Height)
Positive control	170.29 (636)	178.32 (768)	207.58 (3373)	209.74 (3560)
Luminol	170.40 (1528)	178.34 (1596)	207.63 (4984)	209.81 (4510)
LMG	N/R	N/R	N/R	N/R
KM	170.39 (489)	178.34 (436)	207.57 (580)	209.84 (400)
Hemastix [®]	170.38 (1934)	178.28 (1814)	207.52 (1873)	209.59 (1938)
Hemident [™]	N/R	N/R	N/R	N/R
Bluestar [®]	170.31 (3685)	178.27 (2652)	207.36 (4840)	209.46 (4300)
1:10,000	N/R	N/R	N/R	N/R
1:100,000	N/R	N/R	N/R	N/R
1:1,000,000	N/R	N/R	N/R	N/R
Negative control	N/R	N/R	N/R	N/R

KM, Kastle-Meyer; LMG, leuchomalachite green; N/R, no result.

TABLE 5— χ^2 test for consistency results for sensitivity samples with 95% confidence.

Sensitivity	$\nu = 4$ $\alpha = 0.05$ $\chi^2_{4(0.05)} = 9.49$					
	Luminol	LMG	KM	Hemastix [®]	Hemident [™]	Bluestar [®]
Luminol		<i>18.12</i>	<i>12.04</i>	0.00	<i>12.76</i>	8.89
LMG	<i>18.12</i>		2.73	<i>18.12</i>	2.00	4.41
KM	<i>12.04</i>	2.73		<i>12.04</i>	0.11	0.43
Hemastix [®]	0.00	<i>18.12</i>	<i>12.04</i>		<i>12.76</i>	8.89
Hemident [™]	<i>12.76</i>	2.00	0.11	<i>12.76</i>		0.92
Bluestar [®]	8.89	4.41	0.43	8.89	0.92	

The null hypothesis was that the two samples originate from two populations with the same distributions. Numbers in italics reject the null hypothesis at 95% confidence; KM, Kastle–Meyer; LMG, leuchomalachite green.

The Hemident[™] reagent reacted with several of the substances tested. Semen, red onion, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride all showed a color reaction with one or both of the reagents. Semen, red onion, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride all reacted after the addition of the first reagent and would therefore not be mistaken for a possible bloodstain.

The 0.1 M ascorbic acid and 5% bleach samples reacted with a blue/green color on addition of the H₂O₂ as would blood. The 5% bleach samples showed a color change around the margins of the stain, which is not indicative of blood, which reacts on top of the actual stain. The 0.1 M ascorbic acid reacted as blood would for 11 of the 25 samples.

The Bluestar[®] reagent reacted with potato, tomato, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, and 10% ferric sulfate indicated by a blue chemiluminescence upon application.

DNA Analysis

Figure 1 shows the results for the positive control DNA, with the D2 and D19 loci clearly visible. This demonstrates the functionality of the primers and indicates that they would react with any viable DNA obtained from samples exposed to the presumptive tests. These results can be seen in Table 4.

Luminol, phenolphthalein, Hemastix[®], and Bluestar[®] all achieved amplification at both loci tested, which corresponded to the alleles found on the positive control. All four tests gave amplification, although Bluestar[®] claimed that it destroyed DNA (29). Phenolphthalein had a much reduced peak height compared with the other three tests. This is consistent with Hochmeister et al. (24), who found that phenolphthalein reduces the amount of

extractable high-molecular-weight DNA. LMG and Hemident[™] did not achieve amplification.

No DNA results were obtained from any of the dilution series. This could be because there is such a small amount of template DNA that in order to achieve detectable amplification product, it would need several more PCR cycles.

Statistical Interpretation

The results of the χ^2 test for consistency can be seen for sensitivity and specificity in Tables 5 and 6, respectively. The null hypothesis was that the two samples originate from two populations with the same distributions.

The samples that come from populations with the same distributions as each other do not necessarily react with the same substances or at the same rates. Therefore, what one test might react with, another test from a similar population would not react, or if it did it may do so at a different rate. The same distribution comes from the number of substances other than blood that the given reagent will react with.

Conclusion

It is almost never necessary to apply presumptive test reagents directly to dried bloodstain evidence (33,34). However, with extremely small samples, or when testing large areas, it may be necessary to expose the potential bloodstains directly to presumptive tests. Based on this, the best overall presumptive blood test in this study was luminol. It had the greatest sensitivity and specificity. It did not destroy the DNA, and it could be reapplied. Its only drawback is that it must be used in near or complete darkness. Leuchomalachite green was found to be as specific to blood as luminol, but its sensitivity was 10 times less, and it destroyed the DNA. Phenolphthalein had equal sensitivity to most of the other tests, but was extremely unspecific, and the amount of recoverable DNA is reduced when this test is used. Hemastix[™] were easy to transport and use, were sensitive, but not very specific although specificity could be increased if the strips were looked at rather than the reaction on the stain. DNA was recovered from stains exposed to Hemastix[™]. Hemident[®] was specific and sensitive, but destroyed DNA and so cannot be used where subsequent DNA analysis is needed. Bluestar[®] had good sensitivity, but very poor specificity. The need for complete darkness for use further complicates this because even if a stain did not look like blood, it would react in the same way and could be mistaken for blood.

The dilutions of blood did not show any amplification of DNA, but this could be because of the small quantity of template DNA and the low number of cycles of PCR.

TABLE 6— χ^2 test for consistency results for specificity samples with 95% confidence.

Specificity	$\nu = 13$ $\alpha = 0.05$ $\chi^2_{13(0.05)} = 22.36$					
	Luminol	LMG	KM	Hemastix [®]	Hemident [™]	Bluestar [®]
Luminol		13.76	<i>83.08</i>	<i>58.12</i>	14.59	<i>184.17</i>
LMG	13.76		<i>65.01</i>	<i>35.07</i>	18.44	<i>155.28</i>
KM	<i>83.08</i>	<i>65.01</i>		<i>127.26</i>	<i>65.05</i>	<i>144.46</i>
Hemastix [®]	<i>58.12</i>	<i>35.07</i>	<i>127.26</i>		<i>63.69</i>	<i>123.91</i>
Hemident [™]	14.59	18.44	<i>65.05</i>	<i>63.69</i>		<i>145.89</i>
Bluestar [®]	<i>184.17</i>	<i>155.28</i>	<i>144.46</i>	<i>123.91</i>	<i>145.89</i>	

The null hypothesis was that the two samples originate from two populations with the same distributions. Numbers in italics reject the null hypothesis at 95% confidence; KM, Kastle–Meyer; LMG, leuchomalachite green.

Acknowledgments

Thanks are due to WA Products for providing the Hemastix[®], Hemident[™], and Bluestar[®] reagents.

We thank the entire Centre for Forensic Science at Strathclyde University for lab space and equipment.

References

1. Forensic Serology. Available online, <http://faculty.ncwc.edu/toconnor/425/425lect13.htm> (accessed July 2004).
2. Schiro G. Collection and preservation of blood evidence from crime scenes. Available online, <http://www.crime-scene-investigator.net/blood.html> (accessed July 2004).
3. Laux DL. Effects of luminol on the subsequent analysis of bloodstains. *J Forensic Sci* 1991;36:1512–20.
4. Cox M. A study of the sensitivity and specificity of four presumptive tests for blood. *J Forensic Sci* 1991;36:1503–11.
5. Saferstein R. *Criminalistics: an introduction to forensic science*. 8th ed. London: Prentice Hall International (UK) Limited, 2004:320–51, 353–94.
6. Proescher F, Moody AM. Detection of blood by means of chemiluminescence. *J Lab Clin Med* 1939;24:1183–9.
7. Germain O, Miller K. Blood reagents—their use and their effect on DNA. Ottawa: Ottawa (ON) Royal Canadian Mounted Police Forensic Identification Research and Review Section, 1998 (November: Bulletin No. 42).
8. Grodsky M, Wright K, Kirk PL. Simplified preliminary blood testing—an improved technique and a comparative study of methods. *J Crim Law Criminol Pol Sci* 1951;42:95–104.
9. Hunt AC, Corby C, Dodd BE. The identification of human stains—a critical survey. *J Forensic Med* 1960;7:112–30.
10. Higaki RS, Philp WMS. A study of the sensitivity, stability and specificity of phenolphthalein as an indicator test for blood. *Can Soc Forensic Sci J* 1976;9(3):97–102.
11. Frégeau CJ, Germain O, Fournery RM. Fingerprint enhancement revisited and the effects of blood enhancement chemicals on subsequent *Profiler Plus*[™] fluorescent short tandem repeat DNA analysis of fresh and aged bloody fingerprints. *J Forensic Sci* 2000;45(2):354–80.
12. Olsen RD. Sensitivity comparison of blood enhancement techniques. *Ident News* 1985; Aug.:10–4.
13. Ponce AC, Pascual FAV. Critical revision of presumptive tests for bloodstains. *Forensic Sci Comm* 1999;1(2), available online, <http://www.fbi.gov/hq/lab/fsc/backissu/july1999/ponce.htm>
14. Budowle B, Leggitt JL, Defenbaugh DA, Keys KM, Malkiewicz SF. The presumptive reagent fluorescein for detection of dilute bloodstains and subsequent STR typing of recovered DNA. *J Forensic Sci* 2000;45(5):1090–2.
15. Lytle LT, Hedgecock DG. Chemiluminescence in the visualization of forensic bloodstains. *J Forensic Sci* 1978;23:550–62.
16. Albrecht HO. Über die chemilumineszenz des aminoophthalsäure-hydrazids. *Ztschr f Physiol Chem* 1928;136:312.
17. Cox M. Effect of fabric washing on the presumptive identification of bloodstains. *J Forensic Sci* 1990;35(60):1335–41.
18. Gleu K, Pfannstiel K. Ueber 3-aminophthalsäure-hydrazid. *J Prakt Chem N.F* 1936;146:137.
19. Gross AM, Harris KA, Kaldun GL. The effect of luminol on presumptive tests and DNA analysis using the polymerase chain reaction. *J Forensic Sci* 1999;44(4):837–40.
20. Kent EJM, Elliot DA, Miskelly GM. Inhibition of bleach-induced luminol chemiluminescence. *J Forensic Sci* 2003;48(1):64–7.
21. Platt SR. The effects of argon ion laser on subsequent blood examinations. *J Forensic Sci* 1982;27(3):726–8.
22. Messina T. Presumptive blood tests. Available online, <http://www.geocities.com/a4n6degener8/bloodintro.htm> (accessed August 2004).
23. Lee HC, Gaensslen RE, Pagliaro EM, Guman MB, Berka KM, Keith TP, Phipps P. The effect of presumptive test, latent fingerprint and some other reagents and materials on subsequent serological identification, genetic marker and DNA testing in bloodstains. *J Forensic Ident* 1989;39(6):339–58.
24. Hochmeister MN, Budowle B, Baechtel FS. Effects of presumptive test reagents on the ability to obtain restriction fragment length polymorphism (RFLP) patterns from human blood and semen stains. *J Forensic Sci* 1991;36:656–61.
25. Applied Biosystems. AmpFISTR[®] SGM Plus[®] PCR Amplification Kit users manual. Available online, <http://docs.appliedbiosystems.com/pebi/docs/04309589.pdf> (accessed June 2004).
26. UniSTS. Integrated markers and maps. Available online, <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists> (accessed August 2004).
27. Marieb EN. *Human anatomy and physiology*. 5th ed. London: Benjamin Cummings, 2001:651–77.
28. Hemident Blood Reagent. Product information. Available online, http://shop.optimize.com/shop/merchant.mv?Screen=PROD&Store_Code=Red-Wop&Product_Code=4-7812 (accessed August 2004).
29. Bluestar[®] Forensic. Available online, <http://www.bluestar-orensic.com/gb/bluestar.php> (accessed July 2004).
30. Thorpe GH, Kricka LJ, Moseley SB, Whitehead TP. Phenols as enhancers of the chemiluminescent horseradish peroxidase–luminol–hydrogen peroxide reaction: application in luminescence-monitored enzyme immunoassays. *Clin Chem* 1985;31:1335–41.
31. Alvarez de Toledo, Valero R. La reaccion colorante de la sangre con el “leucoverde de malaquita” o tetrametildiaminotriphenilmetano. *Cronica Med* 1935;39:39331–47.
32. Pinker RH. Proceedings of the 20th Annual Convention, Int. Assoc. Identification. 1934;38.
33. Jain P, Singh HP. Detection and origin of bloodstains on various types of cloth immersed in water for a prolonged period. *Can Soc Forensic Sci J* 1984;17(2):58–61.
34. Gaensslen RE. *Sourcebook in forensic serology, immunology, and biochemistry*. Washington, DC: U.S. Government Printing Office, 1983: 112–4.

Additional information and reprint requests:

Niamh Nic Daéid, Ph.D.

Centre for Forensic Science

Department of Pure and Applied Chemistry

Strathclyde University

204 George Street

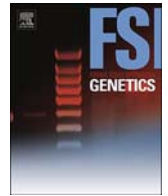
Glasgow, G1 1XW, U.K.

E-mail: n.nicdaeid@strath.ac.uk



Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Presumptive tests: A substitute for Benzidine in blood samples recognition

Cláudia Gomes^{a,*}, César López-Matayoshi^{a,b}, Sara Palomo-Díez^a, Ana María López-Parra^a, Pedro Cuesta-Alvaro^c, Carlos Baeza-Richer^a, Juan F. Gibaja^d, Eduardo Arroyo-Pardo^a^a Laboratory of Forensic and Population Genetics, Toxicology and Health Legislation Department, Medicine School, Complutense University of Madrid, Avda. Complutense s/n, 28040 Madrid, Spain^b Pharmaceutical Sciences Section, Department of Cellular and Molecular Sciences, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru^c Computing Services, Research Support, Complutense University of Madrid, Avda de la Complutense s/n, 28040 Madrid, Spain^d Consejo Superior de Investigaciones Científicas (IMF-CSIC), Archaeology of Social Dynamics, Egipcíacues, 15. 08001 Barcelona, Spain

ARTICLE INFO

Keywords:

Blood
False positive
Benzidine
Tetramethylbenzidine
Leuchomalaquite green
Ortho-Tolidine
BlueStar[®] forensic

ABSTRACT

The nature of the sample in a forensic case is one of the most important factors, since it determines the posterior analysis, helping to define or discard its identity (like blood versus semen).

A presumptive test is a qualitative analysis that allows to identify, or confirm, the presence of a substance in a sample. These determinations usually occur, after a chemical reaction, and a specific colour is produced. A false positive is another substance reacting the same way, producing the expected result.

The aim of this work was to evaluate the most effective presumptive test (with fewer false-positives) when analysing products that could look and behave like blood during a forensic screening assay. Eight different products were tested, like Betadine[®], and four reagents were considered: Tetramethylbenzidine, O-tolidine, Leuchomalachite green and BlueStar[®] Forensic (BlueStar). Each product was tested with the reagents five times – mixed with human blood (3:1), with three different animal blood (3:1), and then unmixed.

Our results indicated that Leuchomalachite green is the most suitable presumptive test, since it was the reagent with less false positives.

1. Introduction

The correct identification of a body fluid allows crucial laboratory tests, like the determination of a genetic profile, a fundamental step in a wide range of investigations [1,2]. One of the most common samples found at crime scenes is the blood stain [1,2].

In order to identify blood, the expert can use different techniques for preliminary identification (screening or presumptive tests). These determinations occur after a chemical reaction, and a specific colour is produced [3].

Several presumptive tests have been described for blood stains recognition [1–3], and one of the most used is Benzidine and its derivative Tetramethylbenzidine (TMB) [4]. Both reagents have similar sensitivity, specificity, and stability [5]. Indeed, Culliford et al., [6] indicate its large number of false positives, for example, due to blood contamination, chemical oxidants, catalysts, or even vegetable peroxidases.

Therefore, the aim of this work was to determine the best reagent for presumptive blood test recognition, with fewer false positives, in

order to substitute (Tetramethyl)benzidine as the first option in blood recognition. To perform so, four reagents will be tested (Leuchomalachite Green (LMG), BlueStar[®] Forensic (BF), and two benzidine derivative, *ortho*-Tolidine (OT), and Tetramethylbenzidine (TMB), on previously described false positives [3,4].

2. Materials and methods

2.1. Samples

Blood human samples were collected from an anonymous and voluntary donor, used for all experiments and positive controls.

Blood animal samples were collected from three death animals: chicken, pig and horse.

2.2. False positives

Products found to give false positives previously [3,4], or with an external appearance similar to a possible blood stain, were tested. Eight

* Corresponding author.

E-mail address: clopes01@ucm.es (C. Gomes).

Table 1

Specificity results for Test b), considering eight commercial products as false positives for blood recognition, after the reaction with four presumptive blood tests (O-Tolidine (OT), Tetramethylbenzidine (TMB), and Leuchomalachite Green (LMG), and BlueStar® Forensic (BF)). (+) positive result; (–) negative result; (?) doubtful result.

Reagent Product	OT + H ₂ O ₂ (3%)					TMB + H ₂ O ₂ (3%)					LMG + H ₂ O ₂ (3%)					BF				
Betadine®	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Wine	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Coffee	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ketchup®	+	–	–	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Nail Polish	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Dish Detergent	–	–	–	?	?	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+
Stain remover	–	–	–	–	–	?	?	?	?	?	+	?	?	?	?	+	+	+	+	+
Bleach	+	–	–	–	–	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+

commercial products were selected, five whose stain might look like a blood stain (Wine, Coffee, Betadine®, Ketchup, Nail polish) and three cleaning products (Dish Detergent, Bleach, Stain Remover).

2.3. Reagents

OT, TMB, and LMG were prepared according to Cox 1991 [1], and BF, according to the manual instructions.

For OT and TMB a turquoise blue colour formation is considered a positive reaction; for LMG a bluish green colour, and for BF a fluorescent blue colour, better observed in a dark room. It was considered a negative result, when the obtained colour was different from the expected one.

To perform each presumptive blood tests recognition, excepting for BF reaction, it is necessary to add H₂O₂ (3%), in the same proportion as the reagent previously added.

The adopted procedure was to perform the presumptive tests in a proportion 3:1 (reagent and H₂O₂ (3%): sample)

2.4. Controls

Positive controls were obtained by applying the reagent in the human blood stain. Performing the same procedure, the negative control was obtained with sterile water.

2.5. Specificity tests

Three distinct analyses were performed in glass support as follows:

Test a) Different animal blood were mixed with each reagent.

Test b) Eight products (“false positives”) were separately mixed with each presumptive test.

Test c) In order to precise the reagents specificity, each false positive was mixed with a positive control sample (1:1). A similar analysis to the previous ones was undertaken, adding each reagent to the sample.

The mixture of the compounds must produce a quick and observable colour change, comparatively to the initial blend colour (see 2.3). The consideration that a reaction was positive or negative was a result of four independent observations. Each essay **a)**, **b)** and **c)** was repeated five times, in order to obtain a significant result ($p < 0,05$).

3. Results

Considering the positive control analysis, all blood samples reacted positively within a few seconds with all of the tested reagents. All negative controls did not yield colour changes.

For Test **a)**, a total of 60 analyses were performed, and 160 analyses were accomplished for each one of the Tests **b)** and **c)**.

On the other hand, by performing five essays for each presumptive test, it was possible to obtain significant results, with $p = 0, 0114$. This value was obtained when comparing 5 positive results *versus* 0 positive results.

Considering **a)** and **c)** essays, all of them produced positive results for all of the four reagents. Table 1 shows the results for Test **b)**.

4. Discussion

Regarding the specificity of presumptive tests for human blood, all reacted positively when in contact with this sample, as well as, when testing human blood mixed with the false positives (Test **c)**). However, the same result was obtained with other animals' blood (Test **a)**), demonstrating the low specificity of these presumptive tests for human blood.

Observing the results for Test **b)**, both LMG and BF has the same efficiency when dealing with false positives, whose aspect could be confused with a blood stain. Nonetheless, the behavior of these reagents is different when tested with cleaning products (Dish detergent, Stain remover and Bleach), where LMG is better than BF.

Concerning OT and TMB, these were the presumptive tests with more false positives detected. The similarity between the structures of these benzidine's derivatives [7,8] can justify that, most of the times, both reagents share the same false positives. However, OT was the only one able to detect “stain remover” as a false positive.

The chemical structure of LMV [9] is quite different from benzidine and its derivatives [7,8]. This implicates that, for a colour change, different conditions are needed, since for the reaction takes place, the sample must interact and modify the reagent structure [7–9]. Such structure differences support the results for LMG, when compared with the other presumptive tests.

Thus, it was possible to conclude that LMG is the best reagent to substitute (Tetramethyl)benzidine in a blood stain recognition, since it was the presumptive test with fewer false positives.

For further research, it would be important to perform similar tests with different trademarks, as well as, with different reagents concentrations. Also, it would be interesting to analyze the behavior of each reagent, supposed to react in the presence of peroxidase, in a cadaver's fluid, since this enzyme is expected to be in lower concentrations.

Conflict of interest statement

None.

Acknowledgment

This work was supported by G/6401400/8000 research project (Santander-Universidad Complutense de Madrid, Spain) for C.Gomes.

References

- [1] M. Cox, A study of the sensitivity and specificity of four presumptive tests for blood, *J. Forensic Sci.* 36 (5) (1991) 1503–1511.
- [2] K. Virkler, I.K. Lednev, Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene? *Forensic Sci. Int.* 188 (1–3) (2009) 1–17.

- [3] G. Delman, V. Manti, S.M. van Ruth, T. van Leeuwen, M. Aalders, Identification and age estimation of blood stains on colored backgrounds by near infrared spectroscopy, *Forensic Sci. Int.* 220 (1) (2012) 239–244.
- [4] S.S. Tobe, N. Watson, N.N. Daéid, Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA, *J. Forensic Sci.* 52 (1) (2007) 102–109.
- [5] D.D. Garner, K.M. Cano, R.S. Peimer, T.E. Yeshion, An evaluation of tetra-methylbenzidine as a presumptive test for blood, *J. Forensic Sci.* 21 (4) (1976) 816–821.
- [6] B.J. Culliford, L.C. Nickolls, The Benzidine test, *JFSCA* 9 (1) (1964) 175–191.
- [7] National Center for Biotechnology Information. PubChem Compound Database; CID = 7111, (2017) <https://pubchem.ncbi.nlm.nih.gov/compound/7111> . (Accessed 19 July 2017).
- [8] National Center for Biotechnology Information. PubChem Compound Database; CID = 41206, (2017) <https://pubchem.ncbi.nlm.nih.gov/compound/41206> . (Accessed 19 July 2017).
- [9] National Center for Biotechnology Information. PubChem Compound Database. CID = 67215, (2017) <https://pubchem.ncbi.nlm.nih.gov/compound/67215> . (Accessed 19 July 2017).



Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forensi

Effect of presumptive tests reagents on human blood confirmatory tests and DNA analysis using real time polymerase chain reaction

Juliana Piva de Almeida^{a,c,*}, Nadine Glesse^{a,b}, Cristina Bonorino^c^a Laboratório de Perícias, Instituto Geral de Perícias do Estado do Rio Grande do Sul, Avenida Azenha, 255, CEP 90160-000 Porto Alegre, Rio Grande do Sul, Brazil^b Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245, CEP 90050-170 Porto Alegre, Rio Grande do Sul, Brazil^c Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, prédio 12, CEP 90619-900 Porto Alegre, Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:

Received 6 November 2009

Received in revised form 3 May 2010

Accepted 14 June 2010

Keywords:

Presumptive tests

Bloodstains

DNA

Serological tests

Bluestar[®]

Luminol

ABSTRACT

Bloodstains often constitute the major physical evidence in crime investigation. Diluted blood invisible to the naked eye can be detected through presumptive tests however such tests can damage samples and prevent further processing such as DNA analysis. In this study, we compared the effects of luminol (prepared according to Weber [15]), Luminol 16[®], Bluestar[®] and benzidine for inhibition in the human antiglobulin test and the human hemoglobin immunochromatographic test and on the total human DNA concentration up to 120 days after sample treatment. Treatment with both luminol solutions and Bluestar[®] still allowed positive results for the immunologic tests, indicating non-interference with human blood confirmatory tests. However, samples treated with benzidine could not be further analyzed by serological tests. Also, DNA quantification showed that 48 h after benzidine treatment, but not luminol or Bluestar solution application, sample DNA was degraded. Luminol 16[®] caused DNA degradation already at 30 days post-application. At 120 days post treatment, all samples treated with any of the agents but not untreated samples had DNA degradation.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Blood detection at crime scenes, in clothes of suspects or victims, or in apprehended objects or motor vehicles, constitutes a major part of physical evidence in criminal investigation, often being decisive for crime elucidation. An adequate collection and preservation of the blood samples is critical to establish associations of the perpetrator with the victim and crime scene. In an attempt to hide evidence, perpetrators can wash bloodstains, making them invisible to the naked eye. Latent blood can then be detected by presumptive tests; however, these should not have an effect on the subsequent tests employed to determine human origin of the blood, as well as DNA analysis.

The literature available on presumptive blood detection techniques is somewhat variable. There is great diversity of experimental conditions, which makes it difficult to compare the use of reagents tested by different authors. For example, in the available studies with luminol, the most widely used presumptive

test, different preparations of luminol and extraction methods have been employed by different groups. Studies focused on DNA analysis using Restriction Fragment Length Polymorphism [9] and STR loci [1,3,5,7,8,11]. All of these studies report that luminol does not degrade DNA. However, Hochmeister et al. [10] have shown that luminol containing sodium hydroxide can affect the performance of the Hexagon OBTI immunochromatographic test after 72 h contact with bloodstains [10]. Bluestar Forensic[®], a more recently developed alternative to luminol, has not had its effects over presumptive tests effects thoroughly studied; the few studies available indicate that it does not affect DNA in the sample [12,14]. However, none of these studies have used a quantitative method. Also, all of these studies had an endpoint of a few hours after Bluestar[®] Forensic or Luminol use [1,3,5,7,8,11–13]. Finally, benzidine, a substance with carcinogenic potential [2,4,6], is used in forensic labs in countries outside the United States, trusting that careful manipulation can prevent its deleterious effects. Very few studies have analyzed the effect of benzidine on presumptive tests. DNA could not be detected by Restriction Fragment Length Polymorphism (RFLP) in bloodstains treated with benzidine diluted in glacial acetic acid [9]. The aim of the present work is to provide a standardized evaluation of the effects of luminol (as prepared by Weber [15]), Luminol 16[®], Bluestar[®] Forensic and benzidine on the inhibition of human antiglobulin test, human hemoglobin immunochromatographic test and DNA retrieval.

* Corresponding author at: Setor de Genética Forense/Instituto Geral de Perícias do Estado do Rio Grande do Sul, Av. Azenha, 255, CEP 90160-000 Porto Alegre, Rio Grande do Sul, Brazil. Tel.: +55 51 3233 6477; fax: +55 51 3233 6477.

E-mail addresses: juliana-almeida@igp.rs.gov.br, julipiva@yahoo.com.br (J.P. de Almeida).

2. Materials and methods

2.1. Samples

All bloodstains were prepared on white cloth (100% cotton) which was cut into squares of approximately 1 cm × 1 cm, previously autoclaved. A 2 µL drop of venous blood (of the same donor for all times analyzed) was added to the squares of cloth and allowed to dry for 48 h. The blood was not subject to any form of anticoagulant. All samples were stored at room temperature, to simulate conditions found in crime scenes. Controls were composed of bloodstains without application of any reagent. For the immunological tests, sample size (*n*) was 20. For the DNA tests, *n* varied between 13 and 29.

2.2. Preparation of reagents

For the benzidine test, 0.2 g of benzidine (Vetec Química Fina Ltda. Rua Pastor Manoel Avelino de Souza, 1021, Rio de Janeiro, RJ, Brazil) was dissolved in 90 mL of water and 10 mL of glacial acetic acid. One drop of benzidine solution 0.2% was added to the cloth. After ensuring that no color was developed, one drop of 3% hydrogen peroxide was added to the stain. Two luminol preparations were tested. One luminol solution was prepared as described by Weber [15]. Luminol was purchased from Sigma–Aldrich Inc., 3050 Spruce Street, St. Louis, MO 63103, USA. The other luminol solution was Luminol 16[®] (Sirchie Finger Print Laboratories, 100 Hunter place, Youngsville, NC 27596, USA), prepared according to the manufacturer's recommendation. Bluestar[®] Forensic (ROC Import Group P.O., Monte Carlo 98005, Monaco) was prepared according to the manufacturer's guidelines.

The method of application for the both luminol solutions and the Bluestar Forensic[®] was the same. Using a spray bottle, the reagents were applied on the samples in a dark room to observe luminescence. The reagents were applied and the samples dried for 48 h (at which time confirmatory and DNA quantification tests were performed), 7, 30 or 120 days (when only DNA quantification testing was performed).

2.3. Confirmatory tests

2.3.1. Inhibition of human antiglobulin test

Each cloth fragment was placed in a test tube with eight drops of physiological buffered saline and left for 48 h at a room temperature of approximately 20 °C. After, a drop of antihuman globulin was added, and the tubes were incubated for 45 min in the same room, then two drops of a suspension of Rh+ human red cells previously sensitized with anti-D antibody (Controcel[®], manufactured by Fresenius Hemocare Brasil Ltda, Rua Roque Gonzáles, 128, Itapeverica da Serra, SP, Brazil) were added. Samples were incubated for another 15 min and then centrifuged at 13,000 rpm for 15 s. Samples with no evidence of agglutination were considered positive for human blood.

2.3.2. Human hemoglobin immunochromatographic test

The human hemoglobin immunochromatographic test (Hexagon OBTI[®]) was purchased from Human Gesellschaft fuer Biochemica und Diagnostica mbH, Germany. Each cloth square was placed in a test tube containing 1 mL Tris EDTA, pH 7.5. After 30 min of maceration, 150 µL of buffer solution was applied to the test strip and the test result was read 10 min after sample addition. Two blue lines, one at the control position (C) and one at the test position (T) indicated that human blood was detected.

2.4. DNA extraction

DNA was extracted from the cloth squares using 300 µL of lysis buffer [100 mM NaCl, 10 mM EDTA (ethylenediaminetetracetic acid), 2% SDS (sodium dodecyl sulphate), 10 mM Tris–HCl (pH 8), 24 µL of 20 mg/mL proteinase K (Invitrogen, Carlsbad, CA, USA) and 48 µL of 1 M DTT (dithiotreitol – Invitrogen, Carlsbad, CA, USA)]. Samples were incubated at 56 °C for 12–24 h. For DNA extraction, 600 µL of UltraPure[®] [phenol/chloroform/isoamyl alcohol (25:24:1, v/v), Invitrogen, Carlsbad, CA, USA) was added, vortexed and centrifuged for 7 min at 15,000 × *g* in a microcentrifuge. The upper aqueous layer was placed inside a Microcon[®]-100 concentrator (Millipore, Billerica, MA, USA) and centrifuged at 500 × *g* until only a few microliters remained. Microcon[®]-100 filtering was repeated twice by adding 400 µL of DNA-free water. Fifty microliters of DNA-free water was added, the columns inverted and the retentate collected by centrifugation at 1000 × *g* for 3 min. The retentate was transferred into a new microtube and stored at –20 °C.

2.5. DNA quantification

Total human DNA was measured in duplicate with Quantifiler[®] Human DNA Quantification (Applied Biosystems, Foster City, CA, USA) in the 7500[®] Real-Time PCR System for Human Identification (Applied Biosystems). The amplification conditions were the ones specified by the manufacturer.

3. Ethics and statistical analysis

This project was analyzed and approved by the Ethics Committee at Hospital São Lucas – PUCRS, number 08/04193. Data were tested for normality of distribution by a Kolmogorov–Smirnov test. Statistical comparisons between each of the groups were made by Kruskal–Wallis one-way analysis of variance (ANOVA) with Dunn's post hoc test. A *p* value of less 0.05 was considered statistically significant. Data analysis was performed with GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

4. Results

The influence of benzidine, luminol, Luminol 16[®] and Bluestar[®] Forensic on the human antiglobulin and immunochromatographic tests can be seen in Tables 1 and 2, respectively. Benzidine applied over bloodstains prevented the human antiglobulin inhibition test (Table 1) in all 20 samples, while all the other reagents did not affect this exam compared to the untreated samples. For the immunochromatographic test (Table 2) samples treated with luminol and Bluestar[®] Forensic produced a positive result in less than a minute after application to the immunochromatographic device. Only two out of the twenty samples treated with benzidine produced a positive result, but only 20 min after application to the device.

The effect of the different presumptive tests on DNA quantification was evaluated at different times after applying the reagents. The results can be observed in Fig. 1. DNA integrity in untreated samples is only altered 120 days after collection (Fig. 1a) (*p* < 0.001). The same result was observed in samples treated with Luminol (Fig. 1b) (*p* < 0.001) and Bluestar[®] Forensic (Fig. 1d) (*p* < 0.001), indicating that these two reagents do not alter DNA amount over time up to this period. Surprisingly, treatment with Luminol 16[®] (Fig. 1c) resulted in considerable DNA degradation already at day 7, with a significant difference at day 30 (*p* < 0.05). Finally, benzidine (Fig. 1e) greatly degraded DNA already at 48 h after treatment.

Fig. 2 compares the effects of the different reagents on DNA quantification at a given time after treatment. At 48 h (Fig. 2a) benzidine resulted in expressive degradation of DNA compared to the control and the other reagents (*p* < 0.001), while neither luminol preparations nor Bluestar[®] Forensic differed from the control, or among themselves. At 7 days, DNA in samples treated with Luminol 16[®] was more degraded not only than DNA in

Table 1

Effect of presumptive tests over the test of inhibition of human antiglobulin.

	Samples (<i>n</i>)	Positive	Negative
Control	20	20	0
Luminol	20	20	0
Luminol 16 [®]	20	20	0
Bluestar [®] Forensic	20	20	0
Benzidine	20	0	20

Table 2

Effect of presumptive tests over the immunochromatographic test for human hemoglobin.

	Samples (<i>n</i>)	Positive	Negative
Control	20	20	0
Luminol	20	20	0
Luminol 16 [®]	20	20	0
Bluestar [®] Forensic	20	20	0
Benzidine	20	2	18

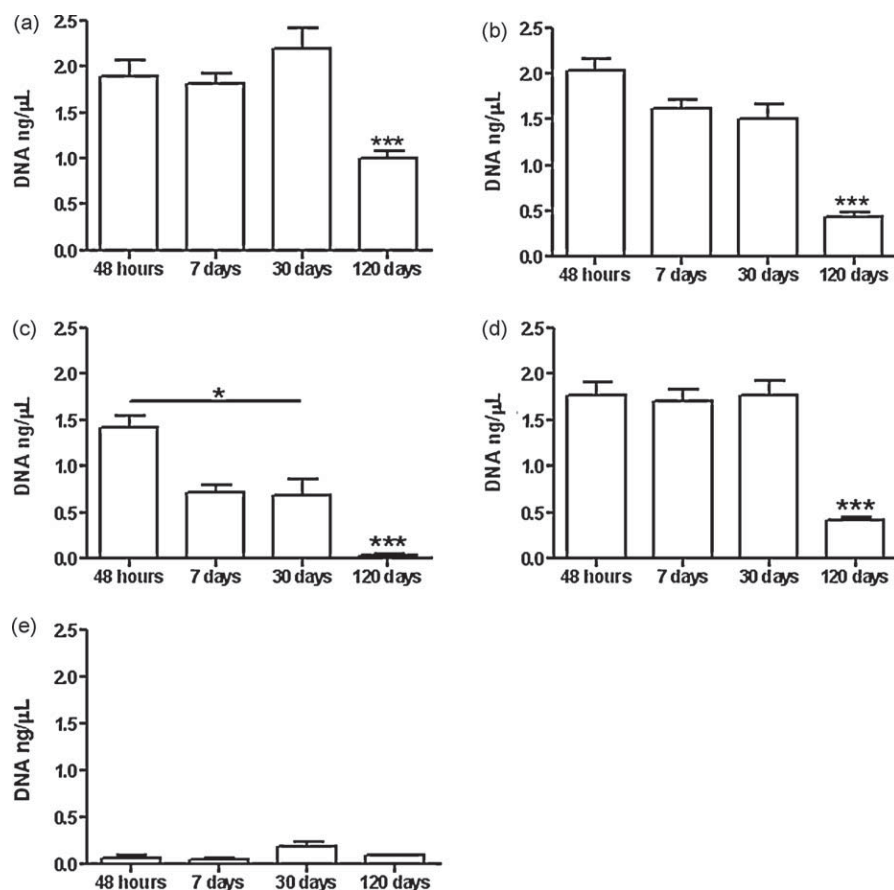


Fig. 1. Comparison of DNA quantification over time in control samples (a), and samples treated with luminol (b), Luminol 16[®] (c), Bluestar[®] Forensic (d) and benzidine (e). KW = * $p < 0.05$; *** $p < 0.001$.

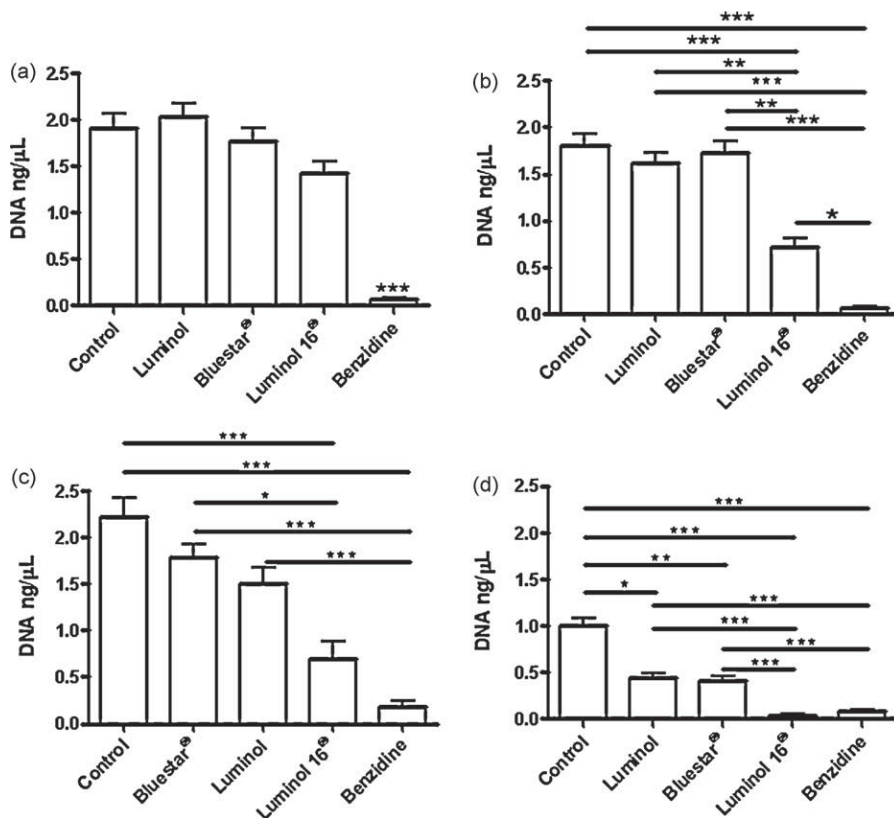


Fig. 2. Comparison of DNA quantification in samples treated with different presumptive tests after 48 h (a), 7 days (b), 30 days (c) and 120 days (d). KW = * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

control samples, but also than DNA in samples treated with luminol and Bluestar® Forensic. At 30 days (2C) the same differences were observed, but at 120 days (2D) all of the tested reagents showed significant degradation compared to the untreated samples ($p < 0.05$). Samples treated with luminol and Bluestar® Forensic did not differ from each other but were different from the control ($p < 0.05$ for luminol and $p < 0.01$ for Bluestar®) as well as from samples treated with Luminol 16® ($p < 0.001$) and benzidine ($p < 0.001$).

5. Discussion

Determination of the amount of human DNA in a stain will indicate the probability of obtaining a full STR profile and is thus critical for investigation of sample identification. Contrary to other findings, this study demonstrates that luminescent presumptive tests affect the amount of the sample DNA after a few months, leading to DNA degradation. However, with extremely small samples or when testing large numbers of samples, it may be necessary to expose the potential bloodstains directly to presumptive tests, and these also save time and money by prioritizing the samples sent for serological tests and DNA analysis.

In this study, the best overall presumptive tests were luminol (prepared according Weber [15]) and Bluestar® Forensic, because they degraded DNA to a lesser extent. Analysis of the Threshold Cycles (C_T) done with the Internal Control PCR (IPC) in the Quantifiler® reactions suggests that presumptive tests degrade DNA, but do not inhibit PCR, since the IPC amplified as expected, presenting a C_T between 20 and 30. The IPC- C_T of controls and treated samples was around 27. Consequently, depending on the amount of biological material available, degradation caused by luminol and Bluestar® is less likely to prevent the ascertainment of a complete genetic profile.

It should be remembered, however, that these tests are used when there are no apparent stains, meaning that very little blood is likely to be recovered, and thus any degradation can be critical for subsequent genotyping. This hypothesis should be pursued by further studies. Also, a comparison with other DNA extraction methods should be performed. The organic method for DNA extraction is the chosen method for scarce and degraded samples in our lab and the forensic community, however a future study should be done with other extraction protocols. Also, in a future study, evaluation of STRs profiles should be performed as a confirmation of Real Time data. DNA genotyping was not performed in this study because there was only quantitative evaluation, but this confirmation is one of the goals of our next studies.

Our results also suggest that, when presumptive tests are performed, serological tests must be conducted in up to 7 days, and genotyping must be done in up to thirty days after the application

of reagents. Finally, benzidine had the most critical effect on blood sample DNA, inducing degradation only hours after treatment, and this result is consistent with Hochmeister et al. [9], data who found that benzidine in acetic acid decreases the DNA by RFLP analysis. Consequently, benzidine should not be used when subsequent DNA analysis is needed.

Acknowledgements

Funding was provided by Secretaria Nacional de Segurança Pública (SENASP) and Instituto Geral de Perícias (IGP) from the State of Rio Grande do Sul.

References

- [1] A. Barbaro, Validation of forensic DNA analysis from bloodstains treated by presumptive test reagents, *International Congress Series* 1261 (2004) 631–633.
- [2] L.J. Blum, P. Esperança, S. Rocquefelte, A new high-performance reagent and procedure for latent bloodstain detection based on luminol chemiluminescence, *Canadian Society of Forensic Science* 39 (2006) 81–100.
- [3] B. Budowle, J.L. Leggett, D.A. Defenbaugh, K.M. Keys, S.F. Malkiewicz, The presumptive reagent fluorescein for detection of dilute bloodstains and subsequent STR typing of recovered DNA, *Journal of Forensic Science* 45 (2000) 1090–1092.
- [4] M. Cox, A study of the sensitivity and specificity of four presumptive tests for blood, *Journal of Forensic Science* 36 (1991) 1503–1511.
- [5] A. Della Manna, S. Montpetit, A novel approach to obtaining reliable PCR results from luminol treated bloodstains, *Journal of Forensic Science* 45 (2000) 886–890.
- [6] C.J. Fregeau, O. Germain, R.M. Fourney, Fingerprint enhancement revisited and the effects of blood enhancement chemicals on subsequent profiler Plus fluorescent short tandem repeat DNA analysis of fresh and aged bloody fingerprints, *Journal of Forensic Science* 45 (2000) 354–380.
- [7] L. Garofano, M. Pizzamiglio, A. Marino, A. Brighenti, F. Romani, A comparative study of the sensitivity and specificity of luminol and fluorescein on diluted and aged bloodstains and subsequent STRs typing, *International Congress Series* 1288 (2006) 657–659.
- [8] A.M. Gross, K.A. Harris, G.L. Kaldun, The effect of luminol on presumptive tests and DNA analysis using the polymerase chain reaction, *Journal of Forensic Science* 44 (1999) 837–840.
- [9] M.N. Hochmeister, B. Budowle, F.S. Baechtel, Effects of presumptive test reagents on the ability to obtain restriction fragment length polymorphism (RFLP) patterns from human blood and semen stains, *Journal of Forensic Science* 36 (1991) 656–661.
- [10] M.N. Hochmeister, B. Budowle, R. Sparkes, O. Rudin, C. Gehrig, M. Thali, L. Schmidt, A. Cordier, R. Dirnhofer, Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood, *Journal of Forensic Science* 44 (1999) 597–602.
- [11] A.C. Ponce, M.A. Seguí, M.M. Feucht, F.A.V. Pascual, Revelado de manchas latentes: efectividad del luminol y evaluación de su efecto sobre el estudio del DNA, *Cuadernos de Medicina Forense* 28 (2002) 33–36.
- [12] S.S. Tobe, N. Watson, N.N. Daeid, Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA, *Journal of Forensic Science* 52 (2007) 102–109.
- [13] K.L. Tontarski, K.A. Hoskins, T.G. Watkins, L. Brun-Conti, A.L. Michaud, Chemical enhancement techniques of bloodstain patterns and DNA recovery after fire exposure, *Journal of Forensic Science* 54 (2009) 37–48.
- [14] G. Tringali, A. Barbaro, E. Insirello, P. Cormaci, A.M. Roccazzello, Rapid and efficacious real-time quantitative PCR assay for quantitation of human DNA in forensic samples, *Forensic Science International* 146 (Suppl.) (2004) S177–S181.
- [15] K. Weber, The use of chemiluminescence of luminol in forensic medicine and toxicology. I. Identification of blood stains, *Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin* 57 (1966) 410–423.

Technical Note

Use of Bluestar Forensic in Lieu of Luminol at Crime Scenes

Lisa Dilbeck

*Scottsdale Police Department
Scottsdale, AZ*

Abstract: Bluestar Forensic, a new luminol-based reagent, was tested in a blood-detection comparison study against luminol. Photographic results were compared and the ease of preparation and the lack of the need for complete darkness for visualization were evaluated. This study determined that Bluestar Forensic has distinct advantages when compared to luminol.

Introduction

For many years, chemiluminescence has been used to detect trace amounts of blood at crime scenes. One popular chemiluminescence product has the generic name of luminol. Luminol is effective in the detection of old, hard-to-see, cleaned-up blood. Luminol has enabled investigators to detect, evaluate, and collect blood not visible to the naked eye. However, some aspects of luminol make its use challenging: It requires almost complete darkness to visualize and photograph. Repeated or constant spraying of luminol increases the amount of fading of the chemiluminescence, and excessive application can create streaking on vertical surfaces and pooling on horizontal surfaces. Luminol must be mixed close to the time of the spraying. Luminol is considered to be a possible carcinogen and requires appropriate personal protective equipment. The process of preparing luminol requires a knowledge of chemistry.

Received September 15, 2005; accepted October 14, 2005

Journal of Forensic Identification
706 / 56 (5), 2006

A new product, Bluestar Forensic¹, is now available as a substitute for luminol. Although Bluestar Forensic is luminol-based and functions in the same way as luminol, its properties make it more convenient for crime scene investigations: It does not require complete darkness, it maintains the same chemiluminescence after each spray, it can be used up to several days after mixing, and it does not require a knowledge of chemistry [1] (Table 1). Most important for investigators is that Bluestar Forensic is convenient to prepare. The purpose of this experiment was to determine whether Bluestar Forensic is a better choice than luminol for use at crime scenes.

Luminol	Bluestar Forensic
Needs almost complete darkness	Does not need complete darkness
Diminished luminescence during second application	Maintains same luminescence during a second application
Laboratory preparation is necessary	Easy to mix in the field
No shelf life after mixing	Can be used for several days after mixing
Not destructive to DNA	Not destructive to DNA

Table 1

Comparison of luminol and Bluestar Forensic.

Background

In 1928, a chemist named Albrecht discovered a chemical that, when placed in an alkaline solution with hydrogen peroxide and a catalyst, would emit an intense blue light, with no heat expelled. This chemical was a precursor to the modern luminol. In 1937, a scientist named Specht used Albrecht's chemical to test a variety of blood-soaked items. In 1951, Grodsky used Albrecht's chemical with sodium carbonate, sodium perborate, and distilled water to detect trace amounts of blood. Grodsky's formula was found to be unstable with the addition of sodium perborate. An improvement on Grodsky's formula was made by Weber (1966), who replaced the sodium perborate with hydrogen peroxide [1]. Weber's formula is in use by police departments today. Luminol is most commonly made by using sodium carbonate, hydrogen

¹ Bluestar Forensic is a product of ROC Import Group, 16 Avenue de la Costa, B.P. 246, Monte Carlo, 98005 Monaco.

peroxide, 3-Aminophthalhydrazide, and distilled water. In 2000, Dr. Loic Blum (University of Claude Bernard-Lyon) devised a new luminol-based formula, which was later named Bluestar Forensic [1]. Although Bluestar Forensic is luminol-based, it is a patented formula and is unavailable for publication [2].

Luminol's action (chemiluminescence) should not be confused with fluorescence. Chemiluminescence requires a catalyst. In the case of luminol, this can be the iron in hemoglobin. Luminol for use in police work is generally bought premade and is then mixed with sodium carbonate and hydrogen peroxide. A disadvantage of luminol is that it can produce false positives when it is used on strong oxidants, some metal ions, and peroxidases. This means that luminescence, although less pronounced, can be seen when luminol is sprayed on copper (or any alloy), bleaches, and horseradish [3]. To overcome this limitation and to help reduce the interference of bleach upon the luminol, it is best to allow the bloodstains to dry thoroughly (giving bleach time to decompose) [3].

Bluestar Forensic was originally produced for hunters. The blood-revealing agent is used to help locate wounded animals. The Bluestar Forensic used for hunters has a pH of 12.6 and therefore is unsuitable for DNA processing of blood. ROC Import Group made Bluestar Forensic for police work to have a pH of 11.5. Because of the adjusted pH, Bluestar Forensic is suitable for DNA STR typing [2]. It has long been known that luminol does not affect the PCR or STR process [4, 5, 6]. Bluestar Forensic does not show DNA degradation, and successful DNA typing has been accomplished up to thirty days after application of Bluestar Forensic [1]. However, for this experiment the focus was on the ease of use of the chemical and not its ability to type DNA; therefore, a mixture of human and equine blood was used and was not typed. For the purpose of this experiment, shelf life, use after preparation, and DNA were not studied.

Methods and Materials

Testing Materials

Six pairs of testing materials were made three months in advance of the actual tests. The first four sets of test surfaces (maple wood, Olefin carpet, vinyl tile, and ceramic tile) were setup in the same manner.

A plastic pipet was used to put impact blood stains on four samples of each test surface. The blood was allowed to dry for 12 days. The blood was then removed from two samples of each test surface with a cellulose sponge that had been soaked in tap water. The blood from the other two test surfaces was removed with a cellulose sponge that had been soaked in tap water containing bleach, hereafter referred to as bleach. The samples were grouped to provide two samples of each surface (one washed with water and one washed with bleach) for each treatment to be tested (luminol and Bluestar Forensic).

The fifth surface was a new blue 100% cotton T-shirt. A shoeprint in blood was put on the front and back sides of the T-shirt. The stains were allowed to dry for 12 days. The T-shirt was then laundered using Purex laundry detergent in a warm/cold wash cycle for 30 minutes. The T-shirt tumble dried for 60 minutes. One side of the T-shirt was to be treated with luminol and the other was to be treated with Bluestar Forensic.

The final surface was a new dark blue CoolMax (polyester) shirt. A shoeprint in blood was put on the front and back sides of the shirt. The stains were allowed to dry for 8 minutes and then the shirt was placed under cold running tap water. The shirt was allowed to dry for 12 days. The shirt was then laundered using Purex laundry detergent in a warm/cold wash cycle for 30 minutes. The shirt was tumble dried for 60 minutes. One side of the T-shirt was to be treated with luminol and the other was to be treated with Bluestar Forensic.

As a control sample, two vinyl sheets were smeared with water containing bleach.

Chemical Preparation

The luminol was prepared according to Scottsdale Police Department's Crime Lab protocol. Ten grams of sodium carbonate and 0.2 g of premixed luminol were mixed with 180 mL of distilled water in a 1000 mL beaker, and then 180 mL of 3% hydrogen peroxide was added. After hand mixing, the solution was then placed into a spray bottle.

The Bluestar Forensic was prepared according to the manufacturer's instructions. Two Bluestar Forensic tablets (one beige and one white tablet) were taken from their sealed pouches

and were added to 175 mL of distilled water in a spray bottle. The spray bottle was closed and the nozzle of the spray was opened for ventilation. The solution was stirred gently by rocking the bottle for approximately 5 minutes until dissolution was noted.

The application of the luminol and Bluestar Forensic was the same. The contents of the spray bottles were sprayed in a sweeping motion on each test sample at a distance of 15 to 18 inches from the object.

Photography

The results were photographed using standard fluorescent photography techniques [7, 8]. All photographs were taken with a Fuji S3 Pro Digital Camera, using an ISO sensitivity rating of 400. Trial exposures were taken prior to the experiment to establish optimum exposure settings. The experiment was conducted in a laboratory environment with dim lights during the Bluestar Forensic tests and a darker (approximately one stop difference) environment for the luminol. The aperture was set at f/6.7 for all of the photographs.

The timed-exposure photographs varied slightly with the initial treatment on the six test surfaces (wood, carpet, vinyl tile, ceramic tile, T-shirt, plastic sheet) (i.e., 3 seconds for the Bluestar Forensic and 4 seconds for the luminol). The timed-exposure for those surfaces during the second treatment was the same for the Bluestar Forensic and the luminol (i.e., 4 seconds).

The timed-exposure photographs for the initial treatment on the CoolMax shirt was extended to 8 seconds for the Bluestar Forensic and 30 seconds for the luminol. No exposures were made during the second treatment.

Results and Discussion

The results of these experiments are shown in Table 2 and Figures 1 - 12. In all cases, Bluestar Forensic outperformed luminol. The carpet showed the best results for both reagents, most likely because the carpet had the best visual bloodstains prior to staining. The vinyl tile and the ceramic tile also showed strong reactions. The most obvious difference between the reagents on these surfaces was the strength of the chemiluminescence in the second spray of the Bluestar Forensic. The majority of the reactions took place in the areas where the blood had been cleaned with only water. It appeared that the bleach removed any traces of the blood from the majority of the surfaces. It was surprising to note that besides the carpet, no false positives were noted between the reagents and the bleach. Both reagents are known to react with materials in bleach. The only reaction to the bleach-cleaned areas was with the carpet; however, no difference in color or intensity could be determined between the water and the bleach areas. This may be because the bleach did not reach some of the deeper embedded bloodstains in the carpet threads. Therefore, in this experiment it was not possible to study Bluestar Forensic's proposed lighter intensity luminescence with false positives.

The washing and drying of the 100% cotton blue T-shirt did not appear to interfere with intensity of the luminescence for luminol or Bluestar Forensic. The dark blue CoolMax shirt showed no reaction to either reagent. This was most likely because the blood had been rinsed with tap water soon after application and then laundered.

Bluestar Forensic's advantage over luminol was most evident in the ability to see its reaction without the need for complete darkness. Although luminol's reactivity might have the same intensity when in complete darkness, in dim lighting, luminol was measurably less intense. Bluestar Forensic is much brighter than luminol in lighted conditions. In crime scenes where establishing complete darkness is not possible, Bluestar Forensic has the distinct advantage.

Test surface	Luminol		Bluestar Forensic	
	Water	Bleach	Water	Bleach
Maple wood	Neg.	Neg.	+	+
Maple wood 2nd spray	Neg.	Neg.	Neg.	Neg.
Olefin carpet	+++	+	+++	+++
Olefin carpet 2nd spray	++	Neg.	+++	++
Vinyl tile	++	Neg.	+++	+
Vinyl tile 2nd spray	Neg.	Neg.	++	Neg.
Ceramic tile	+	Neg.	+++	Neg.
Ceramic tile 2nd spray	Neg.	Neg.	++	Neg.
<i>Control samples</i>				
Vinyl plastic sheet	N/A	Neg.	N/A	Neg.
Vinyl plastic sheet 2nd spray	N/A	Neg.	N/A	Neg.

Description	Bluestar Forensic	Luminol
Cotton T-shirt	+++	++
Cotton T-shirt 2nd spray	++	Neg.
CoolMax shirt	Neg.	Neg.
CoolMax shirt 2nd spray	Neg.	Neg.

Neg. = No luminescence; + = slight reaction; ++ = strong reaction; +++ = very strong reaction

Table 2
Results of experiments.

Conclusion

Overall, Bluestar Forensic was determined to be exceptionally better than luminol in the following areas: ease of mixing, lack of complete darkness, and good intensity after initial spray. For crime scenes, Bluestar Forensic was determined to be a good choice. However, additional testing to determine whether Bluestar Forensic is as sensitive in detecting very dilute concentrations should also be conducted.

Acknowledgments

The author would like to extend her gratitude to Dave Coy, Scottsdale Police Department Crime Lab, for his assistance in photographing the surfaces and reactions; and to Dwane Hilderbrand, Scottsdale Police Department Crime Lab, for his knowledge and assistance with the experimentation and the preparation of this paper.

For further information, please contact:

Lisa Dilbeck
Scottsdale Police Department
Crime Scene Unit
9065 E. Via Linda
Scottsdale, AZ 85258
(480) 312-5445
LDilbeck@ScottsdaleAZ.gov

References

1. Watkins, M. D.; Brown, K. C. Blood Detection. Available at www.bluestar-forensic.com, (accessed July 2005).
2. Lefebvre-Despeaux, Jean-Marc, President of ROC Import Group, Monte-Carlo. Personal communications, 2005.
3. Kent, E. J.; Elliot, D. A.; Miskelly, G. M. Inhibition of Bleach-induced Luminol Chemiluminescence. *J. For. Sci.* **2003**, *48* (1), 64-67.
4. Gross, A. M.; Harris, K. A.; Kaldun, G. L. The Effect of Luminol on Presumptive Tests and DNA Analysis using the Polymerase Chain Reaction. *J. For. Sci.* **1999**, *44* (4), 837-840.

5. Budowle, B.; Leggitt, J. L.; Defenbaugh, D. A.; Keys, K. M.; Malkiewicz, S. F. The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains and Subsequent STR Typing of Recovered DNA. *J. For. Sci.* **2000**, *45* (5), 1090-1092.
6. Laux, D. L. Effects of Luminol on the Subsequent Analysis of Bloodstains. *J. For. Sci.* **1991**, *36* (5), 1512-1520.
7. Staggs, S. *Crime Scene and Evidence Photographer's Guide*, 2nd printing; Staggs Publishing: Temecula, CA, 1999; p 60.
8. Bluestar Forensic User's Manual. ROC Import Group, Monte-Carlo. Available at www.bluestar-forensic.com, (accessed July 2005).

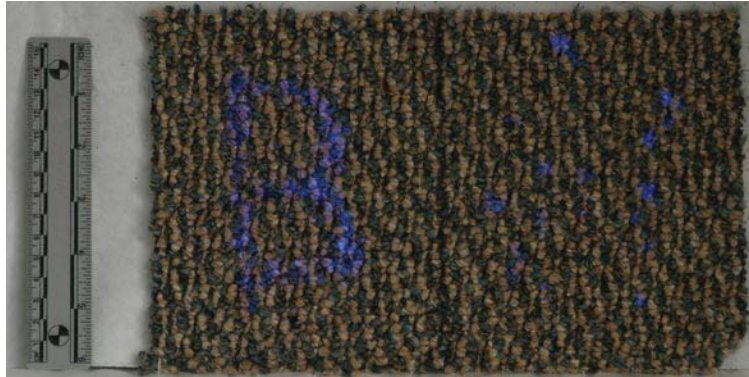


Figure 1

Carpet sprayed with Bluestar Forensic. Left side blood cleaned with water. Right side blood cleaned with bleach.

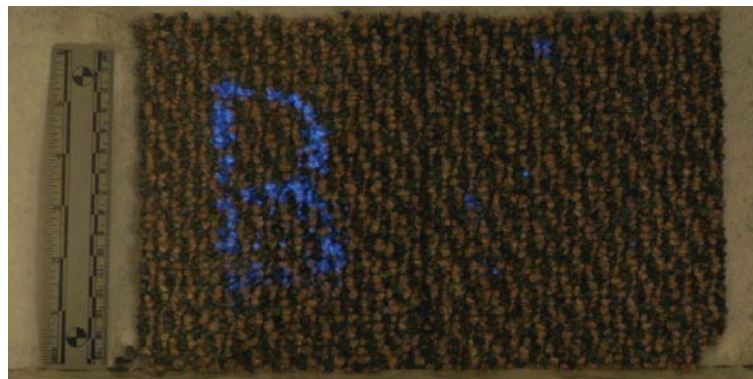


Figure 2

Carpet; second spray with Bluestar Forensic. Left side blood cleaned with water. Right side blood cleaned with bleach.

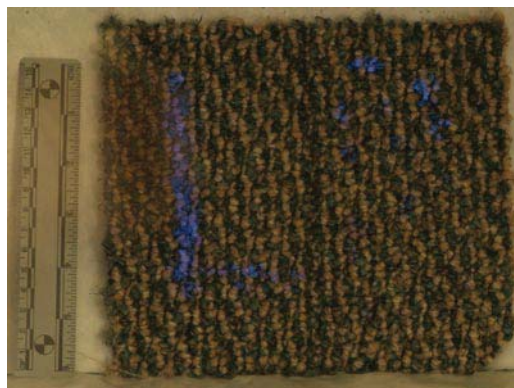


Figure 3

*Carpet sprayed with luminol. Left side blood cleaned with water.
Right side blood cleaned with bleach.*

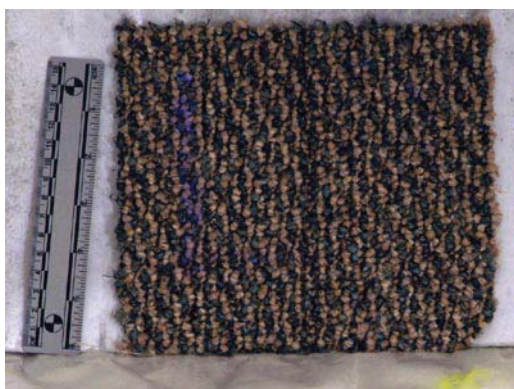


Figure 4

*Carpet; second spray with luminol. Left side blood cleaned with
water. Right side blood cleaned with bleach.*

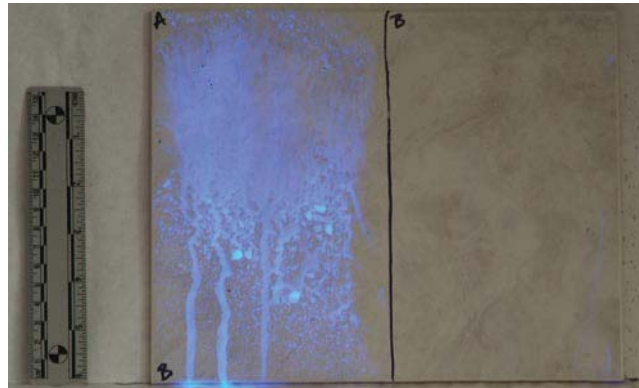


Figure 5

Ceramic tile sprayed with Bluestar Forensic. Left side blood cleaned with water. Right side blood cleaned with bleach.

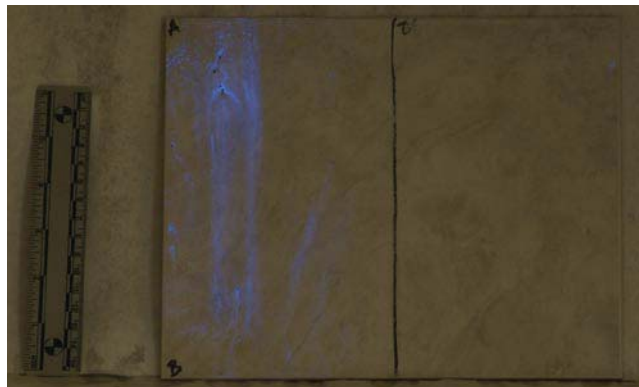


Figure 6

Ceramic tile; second spray with Bluestar Forensic. Left side blood cleaned with water. Right side blood cleaned with bleach.



Figure 7

Ceramic tile sprayed with luminol. Left side blood cleaned with water. Right side blood cleaned with bleach.



Figure 8

Ceramic tile; second spray with luminol. Left side blood cleaned with water. Right side blood cleaned with bleach.



Figure 9

Cotton t-shirt sprayed with Bluestar Forensic after being laundered.



Figure 10

Cotton t-shirt; second spray with Bluestar Forensic.



Figure 11

Cotton t-shirt sprayed with luminol after being laundered..



Figure 12

Cotton t-shirt; second spray with luminol.